The formulation of a pasture seed mixture from a diverse pool of six forage species

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Abstract

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Optimising pasture yield and quality is needed to meet the global food demand. Pasture sward ecosystems that create beneficial diversity and productivity effects will contribute to this. This research used a multi-species pasture mixture experiment to identify optimal seed mixture species combinations under irrigated conditions. This reseach underpins the machanism of linking species mixed pasture properties to the beneficial diversity attributed to community responses. A large-scale diversity experiment used 69 mixtures from six pasture species to investigate the impact of different functional gropus. A simple mixture of combinations of 2 to 3 species from a grass and legume functional group were the key components. On average, mixture communities produced 16% higher biomass yield and contained 64% lower weed biomass than the average performance of the monoculture swards evaluated from 2018 to 2021. Pasture sward responses differed depending on the associated component species, and over time. The model identified several binary and ternary mixture combinations for improved biomass production, weed suppression, and maximising quality; crude protein (CP) and metablizable energy (ME). The highest contributions for herbage biomass were from binary and tertiary mixes of perennial ryegrass (PR), cocksfoot (C), white clover (WC) and red clover (RC), and plantain namely; PR*WC, PR*RC, C*RC, P*WC*RC, PR*WC*RC, PR*C*P and PR*RC*SC. Among those mixture communities, PR*WC and PR*RC maintained the greatest productivity over three years, giving an annual average herbage biomass yield of 13.6 and 15.2 t/ha, when no nitrogen fertilizer was applied. The mixture components in the ternary mixture (PR*WC*RC) produced an annual biomass yield of 15 t/ha. The mixture effect on weed suppression was strong and existed in several mixture communities even though increasing species richness continuously reduced unsown species biomass. Diversity contribution to the nutritional composition of herbage material was not beneficial, but the species' relative abundance in the mixture improved the herbage quality. The optimal mixture combination was PR*RC at the ratio of 50:50 sown seeds equivalent to 12.6 : 13.4 kg/ha (26 kg/ha) viable seed rate. This gave a mean herbage biomass yield per regrowth

cycle of 1.98 t/ha (16 t/ha/yr), with a mean weed biomass of 0.13 t/ha/yr, 10.8 MJ ME/kg DM and 20.7% protein content.

Further, the ternary mixture PR*WC*RC at the proportion of 45:11:44 equivalent of 11.3 : 0.8 : 11.8 kg/ha (24 kg/ha) had a mean yield per regrowth cycle of 1.97 t/ha (16 t/ha) DM, 0.8 t/ha weed yield, 10.8 MJ ME/kg DM and 20.8% protein. This ternary mixture combination at the ratio of 50:15:35 was equivalent to 12.6 : 1 : 9.4 kg/ha (23 kg/ha) and produced a mean yield of 1.95 t/ha (16 t DM/ha), 0.48 t DM/ha weed biomass, 10.1 MJ ME/ha and CP 20.2%. These higher productive mixtures were from legume and non-legume functional groups, which improved the pasture herbage biomass yield and quality compared with their monocultures.

The sown species proportion of swards differed over time based on the functional species composition of the mixtures. Among the monoculture swards, grasses maintained a higher sown species proportion than legumes or plantain. On average, mixture communities suppressed the weeds, and the suppressive effect was increased with the increasing number of species in the mixture. PR*RC binary mixture maintained a higher sown botanical composition proportion over the 3 years than PR*WC. Modelled Relative Growth Rate Differences (RGRD), with the sown proportion, revealed that the equal proportion of the component species in the identified ternary mixture (PR:WC:RC) balanced the competitive growth and maintained the sown proportion until the 3rd year after establishment. This deviated from the optimal seed proportion that maximised the sward yield responses.

Further, this experiment identified that the reason the sward yields differed was investigated in the third year and showed this was due to the quantity of fraction canopy light interception. Overall, species mixed pasture swards intercepted a higher fraction of canopy light (PAR). Average pre-grazing fraction light interception value of swards (2020/21) across the monocultures and two species mixture commmunities was modelled to the initial sown species component proportion. This quantified the diversity contribution of addtitonal fraction of canopy light interception. This was higher for PR*WC (0.47) than for PR*RC (0.10). The higher fraction of light interception contributed to the diversity attributed to the higher herbage biomass in PR*RC than PR*WC. The highest pre-grazing fraction of light interception and intercepted PAR energy was in summer/spring when temperature and available light levels were highest. However, the positive contribution from RC over WC appeared in autumn when the fraction of light interception from white clover may have been compromised by its shallower roots. In the third year, radiation use efficiency (RUE) of swards differed based on the functional species in the mixture community. Grasses had a lower RUE than clovers. There was a positive relationship between RUE and temperature for monocultures and binary mixtures, especially PR*WC and PR*RC.

This research suggests farmers in irrigated conditions should sow a mixed pasture of 2-3 species that comprise a grass and a clover. The PR:RC mixture (50:50 = 12.6 : 13.4 Kg/ha) and PR*WC*RC in both proportion settings (45:11:44 or 50:15:35 = 11.3 : 0.8 : 11.8 kg/ha or 12.6 : 1.0 : 9.4 kg/ha) improved the pasture herbage biomass yield and quality over time compared with their monocultures. These mixtures provided diversity response that persisted and optimised biomass yield with minimal weed content and maintained the botanical composition which inturn optimised herbage quality.

Keywords: cocksfoot (*Dactylis glomerata* L.), diversity, grassland, perennial ryegrass (*Lolium perenne* L.), plantain (*Plantago lanceolata* L), red clover (*Trifolium pratense* L.), subterranean clover (*T. subterraneum* L.), white clover (*T. repens* L.), yield, monoculture, binary mixture, ternary mixture.

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List of Abbreviations

ADF -Acid Detergent Fibre
AWC -Available Water Content
AL -Annual Legume
ASMD -Actual Soil Moisture Deficit
$B_{1,2,3,4}$ -Influential coefficient value (Regression)
C/CO -Cocksfoot
°C -Degree centigrade
Cu PAR_i -Cumulative intercepted PARi
CEC -Cation Exchange Capacity
˚Cd -Degreedays
Coef -Coefficient
CP -Crude protein
CV -Coefficient of Variation
DM -Dry matter
DMY-T -Dry matter yield Total
DMY-W -Dry Matter yield Weeds
DOMD - Digestible Organic Matter Dry weight
em -Emergence
G -Grass function species
g/L -Grams per litre
I -Radiation Intensity below the canopy
ID -Identity Effect
K -Radiation extinction coefficient
l/ha -Litre per hectare
LAI -Leaf Area Index
LAR -Leaf Appearance Rate
M -Abundance
m -meter
mm -Millimetre
ME -Metabolizable Energy
Mg/L -Milligrams per litre
MJ -Mega Jules
N% -Nitrogen percentage
NIRS -Near Infra-Red Spectroscopy
NDF -Neutral Detergent Fibre
NDVI -Normalized Differences in Vegetation Index
NZ -New Zealand
PAR -Photosynthetically Active Radiation
PAR_i -Fraction Light Interception
P/PL -Plantain
P -Probability
P_i -Initial abundance
PET -Potential Evapotranspiration
PeL -Perennial Legume
PR -Perennial ryegrass
PRESS -Predicted Error Sums of Square
R -Intercepted Radiation
R_o -Incident solar radiation
R/R_o -Intercepted fraction radiation
RGR -Relative Growth Rate
RGRD -Relative Growth Rate Differences

R_o -Incident radiation
RC -Red clover
PAR -Photosynthetic Active Radiation
PSWD -Potential Soil Water Deficit
R/R_o -Fraction light interception
RUE -Radiation Use Efficiency (g/MJ)
R^2 -Coefficient of determination
S -Deviation between predicted and observed value
SE Coef -Stand Error of Coefficient
SC -Sub-clover
SE -Standard Error of Mean
SMD -Soil Moisture Deficit
TDR -Time Domain Reflectometry
t -Time
Tt -Thermaltime
t/ha -yield
T_b -Base Temperature
T_{max} -Maximum temperature
T_{min} -Temperature Minimum
T_{mean} -Temperature mean
Tt -Thermaltime
VIF -Variance Inflation Factor
VWC -Volunteer White Clover
WC -White clover
Y -Yield
Y_1, Y_2 -Final biomass yield of species 1 and 2
y_1, y_2 -Initial biomass yield of species 1 and 2
β/α -Coefficient value

Chapter 1
Introduction

1.1 Pasture diversification in pastoral farming

In 2050, global demand for livestock products is expected to double - growing faster than any other agricultural sector (Rojas-Downing et al., 2017). This future demand could be met through increased green herbage biomass yield, although pasture-based farming systems provide fewer opportunities to improve yield and quality over time than cropping systems. Potentially, pasture production could be increased through pasture diversification to bring out the beneficial diversity effect in the pasture system. Some experiments have shown that diversity improved ecosystem services (Hooper et al., 2005) and the stability of plant community responses, as well as pasture yield and quality. However, species diversity in pasture ecosystems does reveal contrasting results: positive (Bullock et al., 2001; Daly et al., 1996), negative (White et al., 2004) and no effect (Zannone et al., 1983), on herbage production and quality. The primary conclusion drawn from these contrasting results is that certain functional groups of species in the mixture are more important than the number of species (Clark, 2001), and can be a strong driver of resource utilization and improved herbage production (Black et al., 2017; Wood et al., 2015) and stability (Tilman, 1996). In addition to more efficient resource utilization to yield improvement, susceptibility to pests and disease might be reduced (Mitchell et al., 2002). The diverse species of mixed pasture swards to enhance community response is integrating areas of pasture physiology to community dynamics on the ecosystem process at different scales. Recent research indicates that there is strong potential for agronomic mixtures to contribute more to pasture productivity (Distel et al., 2020).

A diverse pasture is expected to provide more balanced feed to grazing animals year-round, allowing animals selective grazing. Selective grazing practice in diverse pastures could lead to healthier animals and reduce animal health costs. Pasture species mixture experiments revealed that diverse pasture mix swards improve the quality of animal products (Sanderson et al., 2007). In mixed-species swards, interspecific variation in nutritive value may increase with higher species diversity because of inherent differences in chemical composition and different stages of maturity in the plant community (Huyghe et al., 2008). The careful selection of species for mixture formulation can improve sward quality. In addition, there may be opportunities to deliberately manipulate the pasture composition to deliver specific product attributes (King et al., 2020), e.g. infant milk formula.

The role of forage mixture complexity in the pasture has not been well explained (Sanderson et al., 2004). If component species draw their growth requirements from different niches, diverse pastures achieve more extensive use of growth resources available in the growing environments. This growth process reduces competition within the community. In species mixed pasture farming, it is common to characterize and select species based on their functional group (e.g. grasses, legumes, and herbs). However, the challenge is determining the right combination or mixture of species to maximising the diversity contribution (known as the mixture effect), currently, definitive guidelines for ideal species combination are lacking. In most experiments, the mixture selection was based on an arbitrary or convenient blends, previous field experience, or trial-and-error. There was no scientific evidence or statistical procedure to determine optimization. These mixture functions are based on component species, and species selection for the mixture community is the key to production. In Europe and America, recent advances in mixture experiments were based on mixture design for optimal seed formulation (Cornell, 2002), mainly carried out under cutting management (Connolly et al., 2009; Ergon et al., 2016; Finn et al., 2013a; Kirwan et al., 2007; Nyfeler et al., 2009b) with no grazed examples. These species mixtures focus on community-based swards and are generally used in intensively managed livestock farming, where feed is cut and carried to livestock in confinement. This is uncommon in New Zealand where pastures are grazed *in situ*.

A pasture ecosystem (natural or manipulated) is a community containing different pasture species that coexist and interact with local environmental factors. It covers a wide range of communities of different types and sizes. The functional benefits may only be appreciated when multiple ecosystem processes are simultaneously considered (Hector and Bagchi, 2007). The diverse pasture ecosystem provides key ecosystem services, including support (water and nutrient cycling), provisioning or supporting service, especially ruminant production system (food production), regulation (climate regulation), cultural (recreational), and biocontrol (source of predatory organisms). Ecosystem functions (natural/managed or intensive) need to be widely investigated to gain a better understanding of how a system can be improved by management techniques and increase system outputs (Finn et al., 2013a; Loreau and Hector, 2001; Tilman et al., 2006). In pasture ecosystems, maintaining the species proportion in the swards and associated agronomic practices is vital to improve the pasture function. These functions are generally influenced by several factors, such as species richness, species evenness and edaphic factors such as soil fertility (Sanderson et al., 2004a). In the early 1990s' researchers began to understand the significance of biodiversity for ecosystem processes and output (Ehrlich and Wilson, 1991). Kirwan et al. (2009) proposed a modelling approach to quantify the species diversity effect on productivity. These approaches support agronomic management practices such as multi-species cultivation. Presently, there is a considerable emphasis on low input resource-efficient agricultural systems that reduce production costs and are environmentally sound (Peeters, 2008). For example, legumes in the mixture are an important component in sustainable intensive pasture production system because (1) they provide N into the system and reduce the artificial application of N fertilizer, (2) improve the crude protein (CP) and neutral detergent fibre (NDF) content (Deak et al., 2007) compared with monoculture grasses and, (3) improve the seasonal distribution of forage (Leep et al., 2002) and carrying capacity of a farm.

Ecosystem functions concerning pasture agronomy are mainly biomass yield, weed biomass, and pasture quality. These are considered beneficial in ecosystem services as an output of pasture function, operating in intensively managed pasture systems. These multi-species pasture swards offer measurable outputs depending on the farm expectation and farming system. Further advantages include greater stability in response to disturbance/grazing, reducing the invasion of other species, greater nutrient retention, and aesthetic benefits (Hooper et al., 2005). Low input and viable, sustainable pastoral farming can be practiced by identifying and utilizing beneficial diversity effects, which is currently topical among pastoral farmers.

1.2 Mixture experiment investigating pasture response relationship; Research Framework

Pasture yield and quality are important information for the pastoral farming sector worldwide. Productivity mainly depends on several factors and could be improved more quickly and at a lower cost by mixing different functional component species in appropriate proportions. The pasture community properties such as functional group/species identity, richness, and relative abundance influence ecosystem functions and the resultant pasture responses, of yield and quality. Monocultures and species mixed swards are often used to evaluate diverse species mixed pasture performance. Combining the pasture species to form a mixture may improve production through the combined effect of different species. Mixture experiments explore the relationship between the response and mixture proportion to determine whether some combination of the species can be considered more useful than others.

The diversity-interaction model identifies mixture performance and the component species contribution to diversity effect (Kirwan et al., 2009; Kirwan et al., 2007). These pasture functions differ based on functional species, relative abundance, and their interaction. The evaluation of pasture seed mixture requires multiple years of experiments that consider annual or seasonal responses to initial proportions in the experimental design. This is required to obtain a reliable assessment of the contribution of species distribution and species shifting pattern. The species component proportion in the seed mixture is especially based on species distribution, soil and climate of the farm, long-term effects, and feedback of previous botanical composition shifting patterns and farmers' feedback (Sanderson et al., 2004). The main expectation of mixing the species in a mixed sward would be exploiting the beneficial diversity effect over time. Two non-exclusive mechanisms are assumed to be the main drivers of the positive diversity effect (Huston, 1997;

Loreau, 1998; Tilman et al., 1997) (1) complementarity and facilitation (2) presence of species/functional group/identity. If the mixture yield is higher than the monoculture, it is considered overyielding, and when higher than the best-performing monoculture it is defined as transgressive overyielding (Kirwan et al., 2007).

1.3 Identified research gap with thesis outline, aims and objectives

Pastoral agriculture in NZ faces several challenges and opportunities that will require research and development to provide an effective solution. Over time, improved quality and quantity of herbage production have been a long-standing problem. Productive agriculture grasslands also require high-quality forage, which is often not considered in biodiversity research (Hopkins and Holz, 2006). Blending available cultivars to identify the potential to exploit the diversity effect is a prerequisite for pasture mixture formulation. The successful practice of formulating pasture seed mixes must be carried out from diverse functional species with different growth characteristics which enables them to utilize the different growth resources from their niche. The wide range of mixture communities must be subjected to a diversity interaction model approach to quantify each component species contribution. This experiment explores all possible combinations of mixtures formulated from grasses, herb, and legume functional groups. The ecological theory states that the biodiversity and underlying mechanism may change over time (Hooper and Dukes, 2004; Tilman et al., 2001). Therefore, multiple years of data collection were required to measure yield, quality, and persistence. The overall aim of this research is to formulate the optimum seed mixture composition from a pool of six pasture species that maximizes the herbage yield, ME, CP content and minimizes the weed biomass. The expectation is the formulated optimal seed mixture community must maintain the productive botanical composition over several years before the need for grassland renewal. Natural grasslands are also frequently converted to intensive diverse species of mixed pasture production systems, but the changes in the ecosystem process are unclear. Maintaining the botanical composition of the sward is an expected characteristic, even though interactions affect the balance of species, and are a critical research area in intensively managed pasture systems. Further this research identifies the beneficial pasture species combination/s from a range of mixture communities for improved pasture function over time. The change in botanical composition was also assessed through their Relative Growth Rate (Keating et al.), Relative Growth Rate Differences value (RGRD), and direct comparison of botanical composition. The modeling of RGRD value to initial sown proportion and associated contour plot shows the most resilient seed mixture composition.

Among the environmental variables, light and temperature are the key climatic factors for irrigated pasture production that affect the sward growth and yield. The sward canopy determines the amount of light intercepted and available for photosynthesis. These determine dry matter production with the conversion efficiency or radiation use efficiency (RUE) calculated from the linear relationship between them (Singer et al., 2011). RUE is a valuable parameter for analyzing biomass production efficiency of different swards, specifically monoculture and mixture yield variation. Other external factors such as temperature and herbage N content, which affect photosynthesis are therefore also expected to affect RUE and are considered in Chapter 6.

The species mixed pasture seed formulation and evaluation of species mixed pasture swards for improved yield may require a wide range of pasture species mixtures to be evaluated for identifying mixtures that enhance light interception and biomass production. In addition, several other factors affect mixture performance; the mechanistic interactions among component species in a mixed sward under no fertilizer condition are also complicated. Some species may also dominate over time at the expense of other species, and sward diversity may be reduced. For example, legumes in a diverse species pasture swards may disappear/decline (Guckert and Hay, 2001), and yield improvement by beneficial diversity effect may be reduced (Carroll et al., 2011). The debatable question is "Do diverse systems improve production and persistence?" Perennial ryegrass and white clover mixed pasture is the most widely cultivated combination in New Zealand (Charlton and Stewart, 1999) and produced the highest green biomass yield over time. Further, availability of a wide range of pasture species, requires investigation for combining abilities for improved yield and quality over time. The experiment reported in this thesis aimed to formulate the optimum seed mixture composition from a pool of pasture species that maximizes the herbage biomass yield, lowers weed biomass and has high ME and CP content.

This thesis is presented in seven chapters. Chapter 2 is the literature review, that covers pasture diversification through the mixing of compatible species for improved production and covers a regression modelling approach and response surface methodology for optimization of component species. Specifically, it outlines (a) description of the response surface methodology for pasture seed mixture formulation and (b) the diversity-interaction model approach which identifies suitable species for mixture formulation and quantifies component species' contribution to pasture responses. It also covers (c) response optimization for optimal mixture proportion for improved production and (d) evaluation of mixture productivity determined by herbage biomass yield and quality over time. Chapter 3 covers the materials and methods common to all other chapters. Chapter 4 is the first results chapter. It summarizes herbage biomass yield, quality parameters, beneficial diversity effects and their persistence, and the optimization of component species for improved production. The objectives of this Chapter 4 are,

Objective 1. To Identify the species combinations for improved pasture mixture community responses.

Objective 2. To quantify the diversity contribution (mixture effect) of pasture swards.

Objective 3. To assess the stability of those responses over time based on interspecific interaction contribution.

Objective 4. To identify the optimal component proportion for improved yield and quality attributes.

The main aim of Chapter 5 is to understand the mixture component species dynamics over time. The description and analysis of botanical composition changes were based on different approaches, such as the RGR, and RGRD comparisons, especially for binary mixtures and direct comparison of botanical composition. The modelling of RGRD values was used to identify the most resilient mixture combination of component species. This chapter further, investigates the effect of intercepted light energy and species-specific relative growth rate on the composition dynamics of binary mixture combinations. The objectives are.

Objective 5. To compare the inter and intra mixture productivity (herbage biomass) based on net RGR of component species.

Objective 6. To quantify the botanical composition dynamics of binary mixtures (Relative Growth Rate Differences modelling).

Objective 7. To Identify an optimal component proportion to maintain the botanical composition of ternary mixture (PR*WC*RC- DI modelling for RGRD).

Objective 8. To quantify the effect of the Relative Growth Rate of species as a mixture component species property and intercepted light energy on botanical compositional (RGRD) changes.

Physiological explanations of species mixed pasture swards for improved light interception and biomass production are described in Chapter 6. This chapter evaluates light use efficiency of monocultures and the superior species mixed pasture swards identified in Chapters 4 and 5. The emphasis is on light measurement which was only done in Year 3 once the superior swards had become apparent. It also quantifies the temperature effect on RUE.

Objective 9. To quantify the canopy fraction light (PAR) interception of swards.

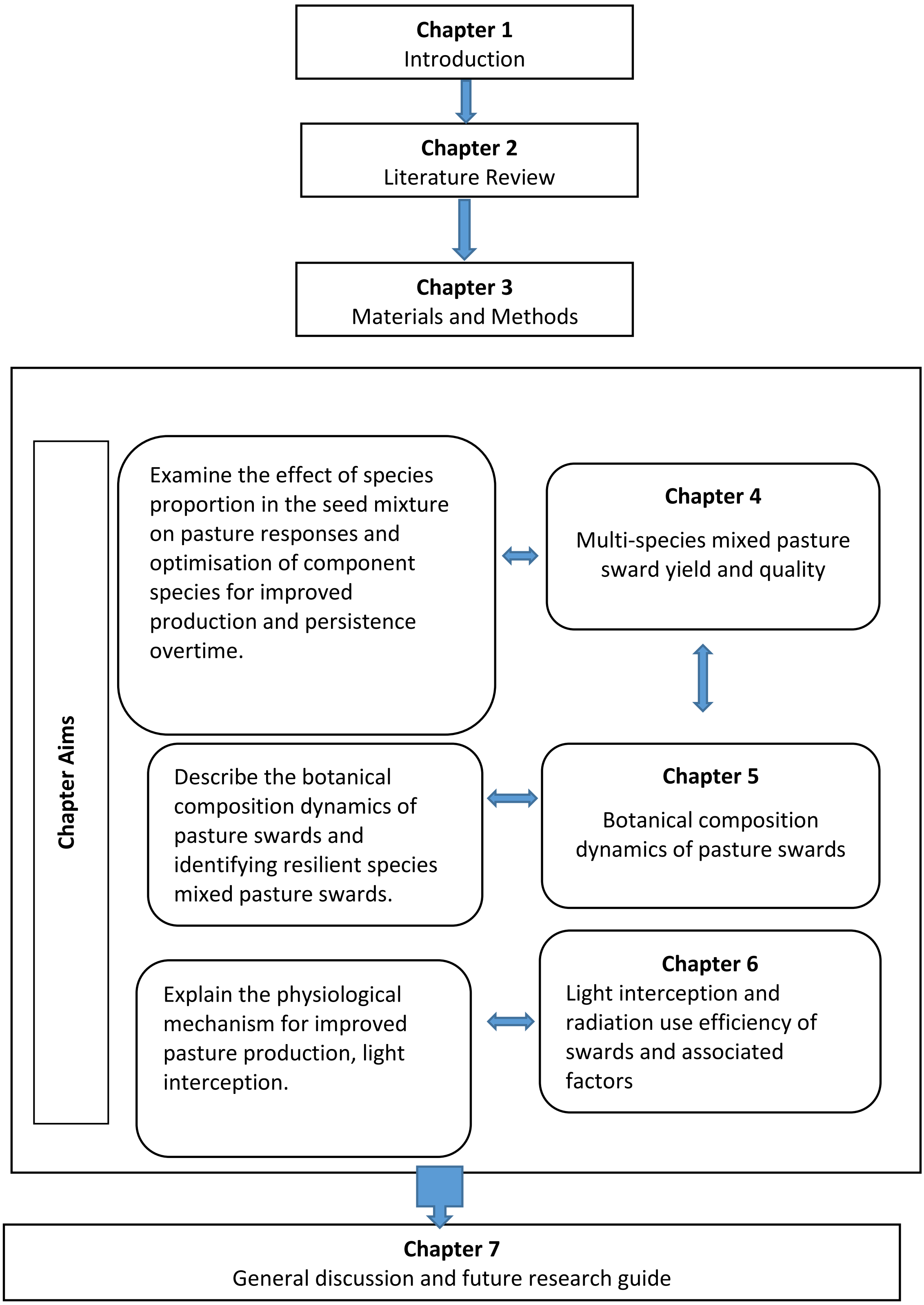
Objective 10. To quantify and compare the RUE of swards including functional species.

Objective 11. To quantify the temperature effect on RUE of selected pasture swards.

Objective 12. To quantify the identity and diversity contribution of species mixed canopy for fPARi, and RUE of swards.

Chapter 7 is the general discussion. This chapter discusses the findings and underlying mechanism (ecological and physiological) for improved sward community responses, supported from other relevant research outcomes for improved sward persisted responses overtime.

Thesis Structure: Diagnostic representation of thesis structure related to chapter content leading to achieving the main aim of the thesis.



Chapter 2
Review of Literature

2.1 Pastoral farming in New Zealand

The primary production industries in New Zealand have rooted themselves as the key economic tree that branches in several directions such as beef and lamb, dairy, deer, goats, pigs, forestry, horticulture, cropping and viticulture. Among the agriculture sectors, pastoral farming has played a significant role in New Zealand's economy (Moot et al., 2009) for over 160 years, and New Zealand has become a leading agricultural product exporter in the world. More than 90% of agricultural outputs are exported, contributing 59% of NZ's export earnings. In the year ended March 2021, this industry's Gross Domestic Product (GDP) accounted for 12.8 billion NZ dollars (Granwal, 2021). Farming covers 13,500,000 ha of New Zealand's total land area (Ball, 2020). Pastures and forage crops are the main feed (95%) for livestock in NZ farms (Hodgson et al., 2005), and the success or failure of pasture production mainly dominates the economy. Thus, it is important to prioritise optimisation of pasture productivity, as the success or failture of forage output drives both the pastoral farming sector and related industries. Pasture diversification has been prioritized to improve farming practices, through the use of improved pasture and animal species. Multi-species pastoral farming practices are facilitated by the commercial availability of many pasture species and cultivars to farmers in New Zealand (Charlton and Stewart, 1999).

2.2 Pasture diversification

Diverse pasture species mix swards are gaining popularity over monocultures and are implemented under the regenerative agriculture concept. The current interest in cultivating diverse pasture may be due to the sustainability or persistence in production, low-cost, and environmentally friendly practices (Jaramillo et al., 2021). The contribution of plant species diversity to productivity and other ecosystem functions is controversial in ecology (Loreau and Hector, 2001). However, increasing diversity in grassland can have a range of effects on eco-physiological processes depending on species composition and environmental conditions. It was also often observed that diversity increased productivity and stability in natural grasslands. The beneficial effects are because of (a) one or few productive species (higher monoculture performance) (Huston et al., 2000), (b) positive interactions among species due to complementarity (niche partitioning for growth resources) or facilitation (Tilman et al., 1996a), (c) a combination of both (Loreau and Hector, 2001). In complementarity relationships, combining species contributes more to the pasture function than in a monoculture alone. NZ summer safe and irrigated pasture production systems are dominated by a perennial ryegrass-white clover mixture. European grassland biodiversity research has revealed that the contrasting effect of plant biodiversity on forage yield could be positive (Barker et al., 2003; Daly et al., 1996) have no effect (Zannone et al., 1983) or be negative (White et al., 2004). The output of some experiments revealed that combining the functional species primarily contributed to diversity productivity (beneficial pasture sward community responses- improved yield, lower biomass and improved nutritional composition) (Clark, 2001). The functional trait diversity and composition in complex forage mixtures can strongly drive resource utilization and yield (Wood et al., 2015). For example, mixing legumes and herbs with grasses has increased primary production (Nyfeler et al., 2009b). Deak et al. (2007) also found that complex mixtures could give more consistent net returns (positive sward community responses) than simple mixtures or grass monocultures. Pasture/diverse pasture swards differ with season and location/district (Baars and Waller, 1979). Harris (2001) proposed three basic principles regarding diverse pasture seed mixture formulation. Those are,

1. It matches species and cultivars to the respective environment where they are going to be sown. This depends on the species' response to temperature, water, and nutrient availability. To have a successful pasture mixture, it must be successfully established and grow in the respective environment.

2. Provide early cover by including species that can quickly occupy the ground, inhibiting the establishment of weeds. This highlights the importance of selecting pasture species with traits that confer a competitive advantage early in development, so they can compete with weeds or slower growth weeds exclude weeds from the community.

3. Select a diverse mixture of species adapted to a broad range of climatic conditions and ecological niches. This reduces the uncertainty regarding competitive exclusion after sowing. Diverse species or cultivars in the seed mix increases the likelihood that at least some species will successfully be established within the available niche and thrive.

2.3 Pasture mixture experiments

Human activities have been changing the environment on a local and global scale. Grassland is not an exception. It has led a dramatic transformation in the composition of pastoral ecology by introducing and improving species and altering species composition. These changes have raised widespread concerns about their effects on sward community responses. Human-involved and most intended positive synergistic interactions in species mixture experiments have been recorded worldwide, including in New Zealand (Connolly et al., 2009; Finn et al., 2013a; Kirwan et al., 2014). Pasture species mix swards have achieved sustainable intensification of temperate-based agricultural production and increased forage production (Tracy and Sanderson, 2004), even in harsh environmental conditions (Pembleton et al., 2015). The key to achieving positive yield benefits is to formulate an optimal seed mix of selected functional species. The presence of one species that enhances the growth, survival, and reproduction of neighbour species, provides beneficial community outcome.

The traditional method of formulating pasture seed mixture provides a limited ability to identify the optimum mix of component species. The formulation of pasture seed mix is based on the total amount of the mix and does not consider the individual proportion of each component species. Diverse sward seed mixture formulations are based on competition studies, and two types of statistic design are commonly used (1) additive series design - increases the relative abundance of one species, and the other is kept constant (2) replacement series design – consists of a number of binary mixtures and monoculture of each of which the overall abundance is kept constant (Harris, 1968; Parry et al., 1994; Stevens and Hickey, 2000).

Recent advancements in biodiversity and ecosystem function research accelerated at the beginning of nineteen 1990s and underwent a fast evolution towards the extension of the modelling approach in mixture experiments. Scheffé (1958) made a strong foundation in mixture experiments through systematic studies, even though Quenouille (1953) first discussed the theoretical framework. Mixture experiments deal with typical multiple regression models where the responses consider relative proportions of the component species in the mixture. In the regression model, components take place in the equation as either a straight regression mixture component or an interaction component of each mixture in regression. The “regression mixture model” (similar to a normal regression) explores characteristics of component species and community properties to enrich knowledge about functional groups/species and community properties. The “Regression interaction model” (cause of the effect on a response) quantifies the degree and strength of any interaction operating in the pasture system for formulating seed mixtures to maximise yield and quality.

Statistical modelling in a mixture experiment aims to model the blending surface to predict the response for any mixture component, singly or in combination. This is based on fitting Multiple Regression models with the intercept set to zero, canonical analysis (multivariate) to determine the shape of the fitted response, and the response surface system. Testing the model's adequacy (lower Residual Mean Squares) would also be an essential part of the statistical procedure in a mixture experiment (Bondari, 2005).

The design factor in the mixture experiment is the proportion of component species in the blend on the response variable. This is based on a modelling approach. Standard mixture experiments include simplex-lattice and simplex-centroid designs (Scheffe, 1963). Cornell (2002) introduced the simplex and axial design's interior point. The simplex-centroid design is composed of mixtures with an equal proportion of components in the mixture. These lay in the middle proportion of all other mixtures in the design graph (see Figure 3.2). This gives a choice to select desirable mixture blends, rather than a monoculture, which have the tendency to alter the response depending on the proportionality of individual components in the mix. Optimal mixture design estimates component proportions for response maximisation or target response (Pal and Mandal, 2012; Pal et al., 2011). Optimization is the final step of mixture formulation.

2.4 Overview of some pasture mixture experiment

This section gives the general idea of pasture mixtures formulated overtime to improve the herbage biomass production and mixture component species used for seed mixture formulation. Despite the considerable number of pasture mixture experiments carried out in Europe, America, and New Zealand, many uncertainties remain in the value of diversity. The history of pasture mixture experiments from 1932 revealed that enhanced biomass yields were obtained from low diversity, especially from binary mixtures (Sanderson et al., 2004). Among the diverse pasture establishment practices, perennial ryegrass and white clover have been a primary mixture in almost all climatic conditions due to the wide adaptability of these species.

Chapman et al. (2018) found that there has been no significant variation in relative herbage biomass accumulation among the range of ryegrass cultivars (intraspecific cultivar variation) as monocultures and mixed with white clover within three years after establishment. Besides this, they explained that the strong general presence of clover in the mixture created marked differences in the productive properties of mixtures compared with the monocultures.

Cardinale et al. (2007) did a metanalysis summarizing 44 diversity experiments that revealed that mixtures produced an average of 1.7 times more biomass yield than the species monoculture. The production was increased over time more than their most productive species (transgressive overyielding) in five years. This result suggests that mixtures were more productive than monoculture.

Sinhadipathige et al. (2012) stated that greater daily live weight gain of lambs during summer and autumn resulted from pasture mixtures containing herbs and clovers rather than perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L) mix.

The short-rotation ryegrass, red clover and balansa clover mixture produced higher forage yield and suppressed weeds more than their monocultures at the end of 12 months after sowing (Ryan-Salter and Black, 2012). The mixtures were at different proportions of the above species at two overall sowing densities (20 and 30 kg/ha). The optimization of the identified mixture consisted of 60%

Italian ryegrass, 40% red clover and no balansa clover. The mixture produced a 14.6 t/ha biomass yield which was 4.8 t/ha higher than the Italian ryegrass monoculture.

Another experiment with perennial ryegrass, plantain, and white clover blends was sown at two relative proportions with two sowing methods. The mixtures and alternate rows of individual species were sown. After two years, the optimal mix of 25% perennial ryegrass, 28% plantain, 47% red clover and no white clover gave a higher yield of 19.9 t/ha. After four years, this seed mixture optimized the yield and quality at 0.25 proportion of each species, equivalent to a seed rate of 7.5 kg perennial ryegrass, 5.6 kg plantain, 1.9 kg white clover, and 4.4 kg red clover. The diversity effect was 8.4 t/ha, a greater yield than the weighted average of the monocultures.

Some experiments have revealed that establishment practices influence pasture growth and yield (Hurst et al., 2000; Thom et al., 2011), but the sowing method did not (Black et al., 2021). Black et al. (2017) modelled 19 seed mixture combinations of perennial ryegrass, red clover, white clover, and plantain under irrigation in the Canterbury region, varying from 1 to 4 species and at different relative abundance. The highest annual DM production was from an optimal seed proportion of 25% perennial ryegrass, 47% red clover, and 28% plantain.

Legume components such as white and red clover can benefit pasture livestock systems considerably (Black et al., 2009). One or two legumes in the grass mixture enhance pasture production and maintain the quality of grass monoculture (Springer et al., 2001). Similarly, subterranean clover can also potentially increase pasture dry matter and ME production in late winter/early spring due to its ability to produce high-quality biomass during this period (Ates et al., 2010).

2.5 Species interactions

Different species' assemblage in a community interacts directly and indirectly within their growing environment (Agrawal et al., 2007). Species interactions form the basis for the ecosystem process and drivers of the ecosystem function. Intraspecific interactions occur between individuals of the same species, while interactions between two or more species are called interspecific. Interaction relationships in grassland association play an important role, especially in species richness, stability, botanical composition, the yield of swards, and the performance of individual species (Braakhekke, 1980). Exploiting the beneficial diversity effect is the primary intention of mixing pasture species. The diversity effect depends on an interspecific interaction (Harper, 1977; Loreau, 2000; Trenbath, 1974). Their total contribution depends on the number and contribution of individual interactions. The contribution of particular interactions depends on the strength of the interaction and the relative abundances of the species involved (Kirwan et al., 2009). Positive interactions among species can increase the performance of the community, a process called facilitation. For example, biological

N fixation of legumes facilitate growth of non-nitrogen fixing species. Different species with complementary traits (e.g., rooting depth) can use different resources or niches, providing the community as a whole with access to more resources and making it more productive than its constituent species individually. Selection effects are apparent when highly productive species dominate the mixture due to interspecific competition (Loreau and Hector, 2001). Harris (1968) explained through the competitive studies on ryegrass ('Ruanui' perennial ryegrass and 'Manawa' hybrid ryegrass) that the potential yield increases could occur if the competitive characteristics of each species are understood when formulating seed mixtures. Black et al. (2017) revealed that combining perennial ryegrass + plantain with either red or white clover maximised the yield due to the species identity and diversity effect under irrigated sheep grazing in mid Canterbury. The planned mixing of species to utilize the positive synergistic interaction in different mixture experiments is also recorded in New Zealand and outside (Black and Lucas, 2018; Connolly et al., 2009; Finn et al., 2013a; Kirwan et al., 2014; Myint et al., 2019).

The direct interaction between two species is caused or altered by simultaneous interactions with additional species (Callaway and Pennings, 2000; Levine, 1999; Miller, 1994). Interspecific interactions include both positive (synergistic), neutral and negative (antagonistic) effects on pasture production (Black et al., 2017). Negative interactions among the plants/pasture appears to derive from the need to acquire/compete for resources such as light, water, and nutrients, which are often in limited supply (Goldberg, 1990; Miller and Travis, 1996). Interactions between species, occur as a pair (pairwise interaction), is most common in mixtures. Direct positive interactions may incorporate a range of mechanisms more than direct negative interactions (Callaway, 2007). Interspecific interactions can determine the difference between individual and mixture performance (Black et al., 2017). This difference is termed the diversity effect and represents how the mixture's actual performance differs from the performance expected from monocultures.

2.6 Biodiversity – Pasture diversity

Biodiversity is simply the variety of life and its process, including the variety of living organisms, genetic differences among them, communities, ecosystems, and landscapes in which they occur, plus the interactions of these components (West, 1993). Biodiversity provides a broad consideration at three levels: genetic diversity, species diversity and ecological and community diversity.

The simplified definition of pasture species diversity refers to the number of species present in an area (i.e., richness) and their relative abundance (Example-evenness). Most grassland studies use species richness as a proxy for diversity. Pasture lands for animal production are a grazing ecosystem that includes primary production, species interaction and competition for growth resources, decomposition, and nutrient cycling. These basic ecosystem functions/pasture functions were influenced by climate, soil, disturbance, and species composition. This eco-system can be manipulated to manage the pasture response and persistence in an intensively managed pasture ecosystem (Sanderson et al., 2007) for direct economic benefits.

Diversity function can be manipulated by the number of species (richness), identity and composition of species or functional groups in the community and relative abundance. Functional groups capture all variation in functional trait diversity. Pasture responses such as yield and quality parameters are important in pastoral farming, and sward productivity depends on the species and their community properties. In the multi-pasture system, species fill niches, increasing the chances of including a production species and regrowth after grazing. Furthermore, these mixtures may also be more resistant to climatic stress and recover more rapidly when conditions improve along with high productivity and nutritional quality relative to monoculture swards.

2.6.1 **Species richness**

Species richness is a commonly used index to measure species diversity, and it does not account for the distribution of plant species in the pasture (Sanderson et al., 2004). Biodiversity experiments in grasslands have shown that species richness enhanced biomass production. However, the effect/response is generally saturated with increasing species richness (Hector et al., 1999; Hooper and Dukes, 2004). Pasture species richness often facilitates productivity, stability, and invasion resistance and reduces vulnerability to environmental disturbance (Finger and Buchmann, 2015). Environmental factors such as topography, climate, soil, pests and diseases and management factors such as sown species proportion, fertilizer, and grazing management influence species richness. Most of these factors can be managed except environmental variables, to some extent, to produce uniform pasture production. Among them, maintaining sward species proportion from the initial seed mix proportion for the relatively few highest performing identified species coexistences is prioritized in this study. The plant species-rich community is expected to more efficiently utilize growth resources (niche partitioning) and have positive interspecific interactions (facilitation) (Huston, 1997; Loreau, 2000) depending on the functional groups in the community. It is challenging to differentiate niche partitioning and facilitation in the pasture community, so the mechanism is often referred to as niche complementarity (Loreau and Hector, 2001).

2.6.2 **Species evenness**

Species evenness describes distribution abundance across the species in a community. In a diversity experiment, the interaction effect depends on the species' evenness. At low evenness (close to a monoculture), all interactions may arise from at least one species or a very low relative abundance.

In this situation, the contribution of interactions to the diversity effect would be lower. Species evenness is highest when all species in a sample have a similar relative abundance.

2.6.3 **Stability of pasture response (persistence)**

The stability of pasture sward responses is important in pastoral farming to sustain farm production and profitability. The pasture community responses are yield, quality and proportion of desired species over a long period- consistency and persistence. It can be defined in different ways (Lehman and Tilman, 2000). This definition must include both aspects: consistency and persistence. Pasture yield and quality consistency covers (1) resistance - the ability to remain constant under environmental stress/disturbance (2) resilience/elasticity - the ability to return to normal or equilibrium stage after grazing/disturbance. Persistence is survival time or the ability to remain within an acceptable range. Pasture persistence cannot be considered just as maintaining a fixed botanical composition for a certain length of time. Long-term studies on grassland dynamics have shown species composition and the relative contribution of each population to overall biomass fluctuation from year to year (season to season). However, total biomass remains unchanged.

Poor persistence of permanent pastures is a major issue for the New Zealand pastoral industry. It was ranked as the fourth most important issue affecting New Zealand sheep and beef farms, with farmers defining a persistent pasture as one that lasts for 7-10 years and remains free of weeds (Tozer et al., 2016). Optimizing the yield and quality while maintaining acceptable plant persistence is key for successful management. The factors that influence pasture persistence are sown species and their proportion, soil moisture, nutrients, and climatic factors, particularly temperature and light. Farmers often recognise their pasture swards when there is a reduced biomass yield, quality, or infestation of weeds. The sward ends up with a decline in the population of sown species or expression of a trait within the sown species present in the pasture (Parsons et al., 2010).

DM yield (herbage and weed biomass) and quality (ME and CP) are economically important pasture responses. DM production is the primary driver of productivity (Chapman et al., 2015; Parsons et al., 2011), and is mainly considered for assessing the persistence in most pasture diversity experiments. The challenge is defining the potential yield. A literature review on forage mixture experiments shows that productivity can be maximized at low or high diversity (Picasso et al., 2008). However, several research findings suggest a complex mixture (with more than two species) can simultaneously maximize more ecosystem functions such as temporal distribution of production, persistence, resistance to the invasion of weeds, and tolerance to fluctuating environmental conditions (Sanderson et al., 2004).

A recent study on the effect of pasture renewal on commercial dairy farms revealed that total annual dry matter production was greater in the renewed pasture in the first, second and third years, but in the fourth and fifth years, there was no yield benefit from renewal (Tozer et al., 2015). The result indicated perennial ryegrass and white clover were productive up to three years in that growing condition. Persistence is dependent on the ability of plants to maintain a high and stable tiller density and the ability of individual tillers to maintain live leaves (Hirata and Pakiding, 2001). Moreover, the study showed that an increased perennial ryegrass tiller population was positively correlated with persistence and long-term pasture yield (Edwards and Chapman, 2011). The model component proportion of PR*WC*RC over three years showed that the mixture-maintained PR proportion 0.57, 0.67 and 0.52, in the first, second and third year (Figure 5.1). In addition to that, the response optimisation were in PR: WC (0.5:0.5) and PR:WC:RC (50;15:35), comprised 0.50 fraction of PR in the optimal mixture. This output implies that density dependent PR composition proportion maintained the botanical composition and dry matter yield over time. Another promising biological improvement is the endophyte associated with ryegrass. Endophyte (animal health safe) enhanced pasture persistence has been of value to the New Zealand pastoral industry, with benefits estimated to be greater than $74 million per year (Stewart, 2006).

A key factor for pasture persistence is the botanical composition of diverse pasture mixture through time and specifically whether the herbs (chicory and plantain) and/or legumes (red clover) persist in the mixture. It is crucial to maintain the botanical composition as any botanical shifts can affect forage quality (Belesky et al., 1999). In the grass-legume association, grasses exploit the ability of legumes to introduce N into the system but simultaneously suppress legume growth through competition for light.

Further, research suggests that most legumes are morphologically unable to distribute widely in mixed pastures (Kessler and Nösberger, 1994). Another study revealed that compared with their monoculture, grass-legume mixtures generally provide more consistent forage yield across a wide range of environments (Bélanger et al., 2014; Sleugh et al., 2000). This relative contribution of each population may be affected by differences in growth rate and perennation strategy of the species involved, selective defoliation, grazing system, and soil nutrient status. For example, frequent defoliation/grazing of red clover reduces its biomass yield and persistence (Black et al., 2009).

With respect to pasture persistence, there has been an increased interest in alternative pasture species such as chicory and plantain for sowing in monocultures or combination with perennial ryegrass and white clover. Yield advantages occur in summer dry areas because chicory and plantain are highly productive with deep root systems (Li and Kemp, 2005). The deep-rootedness enables greater nutrients and moisture capture from subsoil profile/lower than is possible with more shallow-rooted species such as perennial ryegrass and white clover (Li and Kemp, 2005). Blending forage herbs in a grass-clover mixed pasture can change the seasonal feed supply and extend the growing season, with greater production occurring particularly in summer and autumn (Pembleton et al., 2015). The extent to which the effects occur will depend on how evenly the sown species are distributed throughout the pasture rather than increasing the number of sown species per se (Sanderson et al., 2004a).

Persistence of annual species should be considered when establishing or renewing pasture with multi-species (Mcivor, 1993; Reed et al., 1989). In long-term pasture persistent studies in the South Island hill country, swards were sown with 25 species monitored over 19 years. Legumes dominated in the first few years of the study, followed by cocksfoot and Caucasian clover in the second decade, and finally, the sward ended by unsown grasses (Scott, 2001). Red clover generally survives only about 2-3 years (Black et al., 2009), and under sowing red clover in the existing pasture may help maintain the yield and quality persistence. Taylor and Smith (1997) suggested that well-adapted cultivars have a genetic potential to persist for up to five years when physiological stress is minimal. Subterranean clover is one of the important annual legumes in New Zealand that is adapted to a dry environment (Smetham, 1995) and produces high-quality forage in late winter and early spring under proper management conditions (Moot et al., 2003a). Sub clover produces a small proportion of hard seeds, which germinate following early summer rains, causing poor seedling survival and population persistence (Sheath and Hodgson, 1989).

The blending of pasture species mixes without considering functional group and component species proportion may result in poor sward performance and persistence. The functional groups of the species used in this experiment were perennial legume (PeL), annual legume (AL), grasses (G) and herbal plantain (P). Functional group species responses were modelled to the sown proportion of the respective functional group. In a pasture mixture experiment, the functional group may provide an opportunity to select functional complementary characteristics of plant assemblage that influence the ecosystem process. The sown composition may shift to a more stable and simpler mixture of species with traits adapted to growth and survival in this system. Tozer et al. (2016) also stated that a complex seed mix with diverse morphological traits might not be able to persist in intensively managed high-input pastures due to suboptimal management.

2.6.4 **Pasture quality**

Increased dry matter yield of pasture is not always directly linked to increased animal production. Pasture quality is another associated parameter with biomass in pasture production. There is a balance between yield and nutritive value because nutritive value decreases as yield increases (Lee et al., 2015; White et al., 2004). It is strongly influenced by species composition that is comprised of different functional groups with different chemical compositions, phenological stages on photosynthetic pathways i.e. C_3 or C_4 pathway (Andueza et al., 2010; Čop et al., 2009). Herb-clover mixtures can benefit animal production more than standard perennial ryegrass-white clover pasture. Feeding of these mixtures increased lambs' live weight, ranging from 11 to 52%, when grazing herb clover pasture compared with the perennial ryegrass white clover mixture during the summer (Hutton et al., 2011; Kenyon et al., 2017). McCarthy et al. (2020) conducted a meta-analysis which revealed that multi-species swards; herb species grown with either a grass species or grass and legume significantly increased milk production with increased mean fat and protein content. This indicated that the multi species pasture swards improved the herbage quality which in turn enhanced the animal production. In contrast, Woodward et al. (2013)found that feeding of herb-clover mixed pasture did not significantly increase milk production.

Somasiri et al. (2015) conducted an experiment to find the effect of three different pasture mixtures on the live weight gain of weaned lambs and found that either plantain or chicory mixture produced more lamb live weight gain than perennial ryegrass-white clover pastures. Golding et al. (2011) also reported lamb live weight increases when feeding different pastures in a nutritional composition study that included old pasture, a new pasture, a plantain-based pasture, and herb-clover pasture.

A nutritive value experiment was carried out using perennial ryegrass, white clover, red clover and plantain (Vreugdenhil, 2017). The identified optimal mixture for maximization of sward quality was in the ratio of 0.43 ryegrass, 0.2 white clovers, 0.37 red clover. Plantain was not included in the optimal mixture, which may be lower in yield in monoculture plots and have lower crude protein (CP) values (Vreugdenhil, 2017). Deak et al. (2007) indicated that the CP concentration of pasture mixture depends on the legume proportion, and NDF concentration was correlated with the proportion of grass. The nutritive value of perennial ryegrass is reduced when the reproductive stem develops in the summer, 9.9 MJ ME/kg DM, 52% NDF, 22.1% CP (Fulkerson et al., 2007), but another study states that ME levels may be as low as 7.6 MJ ME/kg DM (Burke et al., 2002a). Therefore, minimizing the amount of reproductive growth is an important component of pasture management. The nutritive value of a diverse pasture mixture that includes herbs and legumes during the summer may be higher in nutritional quality than the conventional perennial ryegrass-white clover mixtures that often have poor nutritive values during this time of the year (Burke et al., 2002a). Therefore, changes in herbage quality over time were examined and presented in Chapter 4.

2.6.5 **Species selection**

In formulating pasture seed mixtures, knowledge about species agronomy (growth habit, phenology, nutrient requirement), compatibility in the mixed community, soil fertility status, and intended use of pasture should be considered. Farmers generally prefer to renew their pastures with a mixture of species. Species selection depends on which species need to be included in a seed mixture for a particular condition depending on the species' response to temperature, soil nutrients and soil moisture (Stewart et al., 2014). Mixture component species having higher growth rate and early canopy cover of cultivars provide a growth-based competitive advantage, and in turn occupy the ground rapidly and suppress weed growth in heterogeneous environmental conditions. Further, species in the established sward must adapt to grazing rotation systems and pest and disease management practices.

Harris (1968) described that the diversity effect could not be seen in a mixture with similar functional traits. The species selection for seed mixture formulation must have different growth forms to efficiently utilise the growth resources from different niches. Finn et al. (2013a) also reported that mixing plant species with contrasting characteristics (grasses and legumes) produces a larger yield. The expected yield advantage will be from the mixer effect (diversity) under the same level of resource inputs in the growing environment. The diversity biomass productivity categorised as overyielding/transgressive overyielding could contribute to sustainable pastoral agriculture (Foley et al., 2011). Perennial ryegrass is the most commonly used grass species in temperate pasture systems because of its high production and feed quality (Andrews et al., 2007; Langer, 1990). It is often mixed with white clover, the most commonly used sowing valuable legume in New Zealand (Watson et al., 1996). Plantain is also considered in pasture mixtures because of its palatability of leaves to grazing animals, which produces a mineral-rich forage (Stewart, 1996) and has a greater tolerance to drought conditions than ryegrass and white clover mixtures (Nie et al., 2008). The feeding value of plantain is greater when combined with clovers than when grazed as monoculture (Kemp et al., 2019).

However, Sanderson et al. (2013) pointed out that forage species grown in mixtures are unstable and vary over time within three years after seeding, seldom beyond this time. Papadopoulos et al. (2012) also reported that more complex mixtures produce larger yields between 3 and 5 years after seeding, and after that period, some more competitive species may dominate other species over time.

Clover provides a strong basis for addition to pasture seed mixtures. The addition of clover into pasture mixtures has clear benefits for biological N fixation and their high quality, protein-rich feed value for grazing animals (Stewart et al., 2014). The diversity productivity concept in field application to improve production is only possible by selecting species that belong to different functional group combinations, which utilise growth resources from different niche. In recent years, researchers have found benefits in the productivity of diverse pastures in dairy systems, such as increased DM production in the summer compared with a simple ryegrass white clover mixture (Daly et al., 1996; Nobilly et al., 2013; Ruz-Jerez et al., 1991; Tharmaraj et al., 2008; Woodward et al., 2013). When formulating a seed mixture, individual species performance information and performance in a mixture community with other species need to be considered (Harris, 1968, 2001). Therefore, optimizing seed mixtures with careful preselection of species with different functional traits is a potential strategy to promote overyielding (Finn et al., 2013a; Nyfeler et al., 2009b) with associated sward quality.

2.6.6 **Environmental variables on diverse species mixed pasture swards.**

Physiological and growth processes, such as mineral nutrition, canopy development, canopy light interception, photosynthesis, biomass accumulation and partitioning, directly or indirectly influence pasture/crop production, which would be the effect of combined genetic (species), environmental and management factors (Hay and Porter, 2006). Among environmental factors, temperature, light, and soil moisture are the main abiotic variables that influence pasture growth and yield. Light interception and radiation use efficiency (RUE) of crops directly determines DM yield. Higher light interception and RUE can result in greater productivity (Keating and Carberry, 1993). Intercepted PAR from the sward canopy is the most important factor and a fundamental requirement in temperate pasture production. This mainly limits pasture production and differs with season and light availability. Successful sustainable pasture farming to reach an enhanced quality and quantity of biomass production may depend on a mixture of species management to capture light efficiently by a sward community. In addition to DM production, in grass-legume mixed swards, competition for light determines the species' composition (Haynes, 1980) and sward quality. The limiting effect of reduced light interception on crop growth suggests that manipulating canopies to increase available light on the canopy may increase crop yield. Manipulating multi-species sward canopy structure by deliberately selecting functional species in mixed swards/cropping enhances crop or pasture yield.

Radiation

Among the environmental variables, available solar radiation in the growing environment is the first and foremost factor affecting sward dry matter production under non-limiting optimal growth conditions. Research has investigated the relationship between light and growth morphology/ biomass production/ sward quality. Canopy light interception determines the amount of light energy captured by a crop or pasture, which provides the basis for pasture ecosystem function on primary productivity (Rossiello and Antunes, 2012), especially biomass. Pasture sward canopy/ground cover represents the fraction of light interception (Vos, 1995) and provides light energy for photosynthesis and biomass production, directly proportional to the amount of intercepted light (Moot et al., 2021a). The canopy light interception of a pasture sward depends on cell expansion, leaf appearance rate (LAR), tillering properties and canopy architecture (Biscoe and Gallagher, 1977). Canopy formation of species mixed pasture swards generally resembles mixed cropping. In mixed cropping, PAR absorbed by a plant depends on the ability of the individual plant or the canopy to intercept the incident radiation (Hanan and Bégué, 1995). Intra and interspecific variation in growth and morphology on canopy properties influence light interception, which may affect the pasture sward DM production. In addition to biomass production, in a grass mixture, light plays an essential role in the growth of the mixture and species balance (Willey, 1990). Sward canopy potential for light interception is the prime plant physiological process for DM production, generally measured with highly sophisticated or expensive equipment or can be estimated from elaborative models. The theory of radiation interception by a crop/pasture canopy can be expressed as an exponential extinction function of the Leaf Area Index (LAI); Beer Lambert law (Monsi and Saeki, 2005). This is one of the alternative methods for estimating the fraction of light interception, but leaf area measurement needs to be taken first in this method. The exponential function with Beer's law is given in Equation 2.1

Equation 2.1 Light (PAR) interception and transmission calculation (Beer's law equation)

From the Equation, 2.1 iPAR (intercepted photosynthetically active radiation) can be calculated,

$$\mathbf{I = I_0 * e^{-K*LAI}}$$

Where I – the radiation intensity below the plant canopy

Equation 2.2 Fraction light interception calculation from Beer's law equation

I_o – the radiation intensity above the canopy

K – the radiation extinction coefficient, and LAI – the leaf area index.

$$\mathbf{PARi = I_0 (1 - e^{-K*LAI})}$$

Several studies have shown that diverse swards had a denser canopy (compact canopy) than monocultures because of complementary canopy/crown architecture and plasticity (Jucker et al., 2015). Canopy structures that intercept light and changes the spectral distribution may improve canopy photosynthesis depending on canopy properties and environmental factors. Light (spectral) distribution inside the canopy layer in the species mix sward differs depending on the species present (Ligot et al., 2016). Diverse species mixed sward seed formulation for cultivation is a way to manipulate canopy light interception or the light environment in the canopy for improved pasture production. Multi-species sward is a low-cost agronomic practice exploiting the hidden diversity potential for improvement of pasture production. This phenomenon is in accordance with the general prediction of fundamental biodiversity research that more diverse systems use resources more efficiently due to the complementary between species (Forrester, 2014; Loreau and Hector, 2001; Tilman, 1999).

In contrast to the beneficial effect, species mixed sward, complete overlapping of one species by the other leads to unequal competition for light. The fatal consequences of excessive shading dramatically decrease the photosynthetic production of mixtures. Manipulating crop canopies through species selection with an appropriate relative proportion of component species in seed mixture formulation maximises the intercepted sunlight, improving biomass yield and reducing weed infestation (Holt, 2017). To achieve this objective, farmers and agronomists try to manipulate the sward canopy by mixing different functional species to maximize photosynthetic dry matter production and reduce weed competition. This is a generally accepted agronomic practice in sustainable pasture management. Optimisation of canopy structure for the light interception in mixed pasture swards/mixed cropping can enhance crop/pasture yield (Feng et al., 2016). This is because of a higher potential for radiation capture and use than in monocultures, leading to higher productivity (Masvaya et al., 2017; Tsubo et al., 2005). The pasture sward canopy is the primary component of the pasture system for light and photosynthetic storage organ for utilization. The growth rate of crop/pasture is proportional to the intercepted PAR (Luo et al., 2017), invariably correlated with canopy radiation interception and radiation use efficiency.

Radiation interception on biomass production

Under non-limiting optimum growth conditions, pasture or crop yield responds linearly to the amount of solar radiation intercepted (Daughtry et al., 1992; Green, 1987). This concept explains that pasture/crop yield is the function of the components shown in Equation 2.3 (Hay and Porter, 2006).

Equation 2.3 Light interception on herbage biomass production model

$$\mathbf{Y} = \int_{em}^{t} (\mathbf{R_o \text{ x } R/R_o \text{ X } RUE}) \text{ X } \mathbf{HI}$$

Y = above-ground biomass yield, R_o = daily incident solar radiation received, R = intercepted radiation, R/R_o = fraction intercepted, RUE is the overall photosynthetic efficiency of pasture, HI = fraction of dry matter produced, which is the harvested or grazed biomass, t = Time, em = emergence.

Above-ground biomass production of crop/pasture is directly related to the amount of photosynthetically active radiation intercepted by the canopy during its life cycle, re-growth cycle, or based on absorbed PAR (Daughtry et al., 1992; Green, 1987). After canopy closure, under non-limiting growth conditions, light energy availability does not limit photosynthesis (Hay and Porter, 2006). Total above-ground biomass harvested or grazable is an economically valuable component, generally considered for harvest index (HI) calculation; the value for grass is 1. Some researchers considered above and below-ground biomass as the total photosynthetic biomass of plants (Green, 1987). Photosynthetic product/biomass yield can be expressed as the product of cumulative intercepted PAR during the re-growth cycle or crop growth duration (Bonhomme, 2000). Phyto-mass production can be expressed based either on the amount of photosynthetically absorbed or intercepted radiation on plants/pasture canopy, biomass production per unit intercepted light is RUE (Monteith et al., 1977). The key determinant of pasture yield is that a crop/pasture RUE is proportional to its photosynthate assimilation rate per unit of intercepted radiation. Crops/pasture species grown together in mixed systems modify the microclimate, especially the light regime (Tournebize and Sinoquet, 1995). RUE of intercrops/mix pasture swards is generally associated with different variables, including the component functional species, leaf photosynthetic capacity, agronomic practices, pest and disease, precipitation, soil moisture, and soil nutrients. These factors affect a pasture plants' net photosynthetic capacity and the canopy's structure for light interception (Li et al., 2008). A better understanding of the formulation of diverse species mixed swards for efficient canopy formation and light interception may improve pasture production over time, with minimal or zero changes in botanical composition.

Temperature

The temperature is the primary factor driving plant/pasture morphological and phenological development (Hodges, 1991). The rate of plant/pasture growth and development depends upon the temperature surrounding, and each species has a specific temperature range represented by minimum, maximum and optimum. In most cases, plant/crop physiological processes and development rate increase with temperature between the minimum and optimum limit. From an agronomic point of view, matching the pasture species to the temperature of the growing environment is critical for improving production, especially the temperature accumulation over the growth duration. The diverse species mix sward forms different niches, creating a complex ecological process depending on their functional species and exposure to seasonal temperature variation. These component species have different inherent cardinal temperatures, and optimal performance/growth is accelerated with seasonal temperature changes to ensure continuous pasture production. Effects of temperature on pasture species are extensively studied for pasture growth, yield and phenological development. Green leaves (Greener portion) of the sward are important for photosynthesis. Under non-limiting growth conditions, primarily moisture, the production of leaves in pasture legumes is driven by the accumulation of daily mean temperature above base temperature and the phenological development phase (Black et al., 2002; Moot et al., 2000).

Seasonal temperature variation affects pasture production. The plant processes linked with growth and phenological development can be accurately quantified by thermal time or growing degree days (˚Cd). This is the heat unit accumulation on a daily base for the mean daily temperature above a base temperature. It is generally calculated to quantify the effect of temperature on the plant development process (Baars and Waller, 1979; Jamieson et al., 1998a; Moot et al., 2000) but can also be used to estimate pasture growth (e.g., Mills et al., 2006), because is summarises photosynthesis responses to canopy light interception.

2.7 Design and analysis of mixture experiment

The basic concept of a mixture experiment is the system/method for assessing mixtures and their dynamic interaction over time. In species mixed pasture experiments, response factors are relatively biomass yield of each species to the densities of the component species. The mixture models' development dates back to the nineteenth century (Desarbo and Wedel, 2002). Mixture models are part of a group known as the finite mixture models. Since the development of mixture models, several alternatives have been proposed, including the regression mixture model and regression interaction model. Cornell (2002) developed the recent methodology, and the independent factors are the proportions of components in the mixtures used in pasture mixture experiments (Black et al., 2017; Ergon et al., 2016). The mixture experiment allows the building and analysis of simplex lattice and simplex centroid designs. The simplex design is the basic design in the mixture experiment. Each point on the design represents a specific blend of component species in the experiment. The simplex centroid design is applicable when all components have equal proportions (Cornell, 2002). It is a centre point in the design mix in the seed run with an equal amount of all ingredients that are always included in the design point. Axial point blend is the special blend that includes all ingredients between the centre point and vertex. This research setting includes six species comprised of grasses, legumes and herbs. These six species were blended to create 69 mixture combinations in four replicates and thus 276 individual plots, as described in Chapter 3. In the pasture mixture experiment, the botanical composition ratio of the previous year may be used as the initial proportion setting for the consecutive year (Kirwan et al., 2009), and this was assessed during the current study.

2.7.1 **Regression mixture model**

The mixture modelling framework can generally be applied to investigate the relationship between the mixture's response variable and predictor/population. It was first introduced to statistical literature by Quandt (1972), who proposed the use of regression relationships in econometric data. Regression mixture model analyses are generally used to investigate the relationship between a dependent (Categorical or continuous) variable and a set of independent variables based on a particular population or group (Cody, 2006). Regression mixture models and regression interaction models are finite mixture models (McLachlan and Peel, 2000), generally termed mixture models. Regression interaction models quantify the diversity effect operating in a mixture combination (Black et al., 2017; Kirwan et al., 2009). These two models are generally used to efficiently formulate multi-species seed mixtures of different components.

The framework estimates monoculture and mixture performance in diverse mixed pasture communities. Diversity-interaction models (Black et al., 2009; Kirwan et al., 2009) are used for analysing and interpreting data from mixture experiments that explore the effects of species diversity on community-level responses. These models accurately predict the contribution of the component species to the diversity effect rather than measure the overall effect of diversity as in the earlier seed mixture experiments. The general formulation of the linear model as the community-level response (biomass yield, quality) is the sum of Identities, diversity effect (DE) and residuals.

Equation 2.4 Monoculture/Identity contribution on multi-species pasture ecosystem function.

$$\boldsymbol{ID = \sum_{i=1}^{S} \beta_i P_i + \alpha M}$$

The ecosystem function in the mixture is an average of the identity effects of the component species, weighted by the initial proportions of the mixture, represented in Equation 2.4. When monoculture performances of individual species differ, the addition or loss of species will impact ecosystem function. In the absence of the species interactions, the performance of the mixture would be identities and relative abundance of species present and initial overall community abundance. In this experiment, seed mixtures are sown in one overall density.

The coefficient α- reflects the effect of changing initial overall abundance on ecosystem function; M is the overall sowing density of the mixture; βi is the level of ecosystem functioning at average overall sowing density (M), and Pi is sowing proportion. In addition to the individual performance of the monoculture, the diversity interaction provides additional contributions due to interspecific interactions.

Equation 2.5 Identity and diversity contribution on multi-species pasture ecosystem function.

The contribution of pairwise interactions is explained as

$$\boldsymbol{Y = \sum_{i=1}^{s} \beta_i P_i + \alpha M + \sum_{i,j=1}^{s} \delta_{ij}\, P_i P_J + \varepsilon}$$

Where δ_{ij} reflects the potential of two species to interact, and this potential performance is quantified by the relative abundance of the component species P_i and $P_{j,}$ to give the contribution (P_iP_j) of that interaction to the response. The net effect of interspecific interaction on pasture response may be positive or negative. The interaction between the species i and j, δ_{ij} may be positive, indicating complementarity or negative, indicating competitive suppression. The potential additional performance of the mixtures can be quantified through the model's interaction coefficient value. The net effect of positive interspecific interaction in the sward community may lead to overyielding where the performance of the mixture exceeds that expected from the average monoculture performance or higher than best monoculture yield, transgressive overyielding. Ɛ- is a random error term, assumed to be typically and independently distributed with the mean zero and constant variance. The model analysis associated with the Analysis of Variance (ANOVA) tests whether the estimates are different ($p<0.05$) to zero. The non-significant terms in the model are generally removed and re-analysed to improve the model accuracy. The statistically significant mixture model becomes the basis for the response surface graphs. Analysis of Variance results reveals that the predictive estimate for a polynomial regression fit. The response optimisation procedure in Minitab defined the optimal composition of the seed mix for a single response and multiple responses for several mixture proportion variables/species.

The quantifying diversity contributed beneficial pasture responses due to the interaction between species, requires systematic experimental design as described in mixture experiment. The methodology section in Chapter 3 describes the treatment combination and statistical methodology of this experiment.

Chapter 3

Materials and Methods

3.1 Site description

3.1.1 Site location and history

The experimental site was at Lincoln University in New Zealand (43°38'52.15"S, 172°28'3.63"E and 9 m above sea level). The experimental area was 0.66 ha (110 m X 60 m) of flat land in Iversen field paddock (I8). The site was under perennial ryegrass and white clover for eight years prior to this experiment.

3.1.2 Soil characteristics

The soil type is a Wakanui silt loam (New Zealand Classification: Mottled Immature Pallic Soil; United States Department of Agriculture Classification: Udic Ustochrept). Wakanui silt loam soils typically have 0.3 m of topsoil and subsoil horizons consisting of silt to loamy sand to a depth of 2 to 3 m. These soils have a high mineral nutrient content, especially sulphur, and imperfect drainage due to their high density causes mottling below 0.7 m. The available water-holding capacity of Wakanui silt loam ranges from 120 to 180 mm/m (Webb et al., 2000).

3.2 Meteoreological condition

3.2.1 Long-term meteorological condition

The long-term mean data (LTM) (2002-2023) for the experimental site is displayed in Table 3.1. The Canterbury region is categorized as a cool temperate climate with an average annual rainfall of 627 mm spread equally over the year. The annual long-term mean monthly temperature is 11.8 ˚C, ranging from 6.8 ˚C in July to 17 ˚C in January. The mean daily solar radiation ranged from a minimum of 4.5 MJ m^{-2} on a cloudy day in June to a maximum of 22.8 MJ m^{-2} on a clear day in December. Long-term mean monthly Penman Potential Evapotranspiration (PET) usually exceeds the annual precipitation from November to March. With a long-term mean annual PET of 1013.6 mm, this gives a long-term maximum potential soil moisture deficit (PSMD max) of 350 mm.

Table 3.1 Mean monthly long-term data (2002 -2022) for precipitation, and potential evapotranspiration. The average maximum (T_{max}), minimum (T_{min}) and mean temperature, Total solar radiation and windrun obtained from Broadfield meteorological station, Canterbury, New Zealand.

Month	Rainfall (mm)	Tmax (˚Cd^{-1})	Tmin (˚Cd^{-1})	T mean (˚Cd^{-1})	R_o MJ M $^{-2}$d^{-1}	$EP_{(po)}$ (mm)	Windrun (km d^{-1})
January	40	22.3	11.7	16.7	22.5	150	393.9
February	42	22.0	11.6	16.4	19.3	113.8	382
March	43	20.3	10.0	14.9	14.5	87.6	360.6
April	57	17.4	7.1	12.2	9.5	50.6	316.7
May	61	14.9	5.0	9.9	6.0	31.2	308.2
June	61	12.0	2.6	7.3	4.5	18.9	287.5
July	60	11.5	1.9	6.6	5.3	21.9	289.3
August	53	12.7	3.1	7.8	8.0	35.4	320.7
September	32	15.1	4.4	9.8	12.7	61.0	359.8
October	45	16.7	5.9	11.2	17.9	93.4	379.4
November	42	18.9	8.1	13.4	22.1	122.5	384.1
December	50	20.5	10.5	15.3	22.7	136.4	398.5
Annual	627	17.0	6.7	11.5	13.7	923	350.7

3.2.2 Solar radiation and Temperature during the experiment

Mean daily solar radiation and air temperature during the experiment followed the long-term pattern. During the experimental period, the mean monthly air temperature ranged from 6.6 ˚C in July to 16.7 ˚C in January. The highest temperature of 33.7 ˚C was recorded in February 2020 and the minimum of -4.2 °C in June 2018. Monthly mean solar radiation increased from a minimum of 4.5 MJ m^{-2}day^{-1} in winter to a peak in summer. Daily mean solar radiation ranged from 1 MJ/m^2/day in July to 23 MJ/m^2/day in December.

3.2.3 PET, PSMD during the experiment

Soil moisture deficit (SMD) was calculated daily using Equation 3.1. PET decreased to a deficit greater than half of the field capacity (155 mm in top 0.5 m). If the deficit was less than zero, then drainage was considered to have occurred. The water balance assumed a starting deficit of 0 mm on 1 July 2017 and was reset on 1st July each year to calculate the actual soil moisture deficit.

Equation 3.1 Daily soil moisture deficit calculation

SMD (daily) = SMD previous day + Today's PET - Today's rain - Today's irrigation (I)

The water requirement is equal to the difference between potential evapotranspiration (PET), and rainfall plus Irrigation (I). Daily rainfall and potential evapotranspiration (PET) data were obtained from a NIWA climate station at Broadfields, approximately 2 km north of the site. Figure 3.1 shows the actual and potential soil moisture deficit, rainfall, and irrigation from 2018 to 2021. The available soil water content was set at 200 mm and the maximum actual soil moisture deficit (ASMD) was 83 mm/m in the establishment year (03/02/2019). The differences between actual and potential soil moisture were highest in 2019/20 growth season.

Irrigation was applied at approximately 5-10 days intervals over summer and autumn based on the soil moisture deficit. Irrigation aimed to maintain the soil moisture level above 75% of the maximum available soil water content or 150 mm and this was achieved for most of the experimental period. The amount of water applied was measured using rain gauges. The application rate was 15 mm/hour. The field capacity of the experimental site was considered as 310 mm based on previous measurements (Black and Lucas, 2018; Brown, 2004).

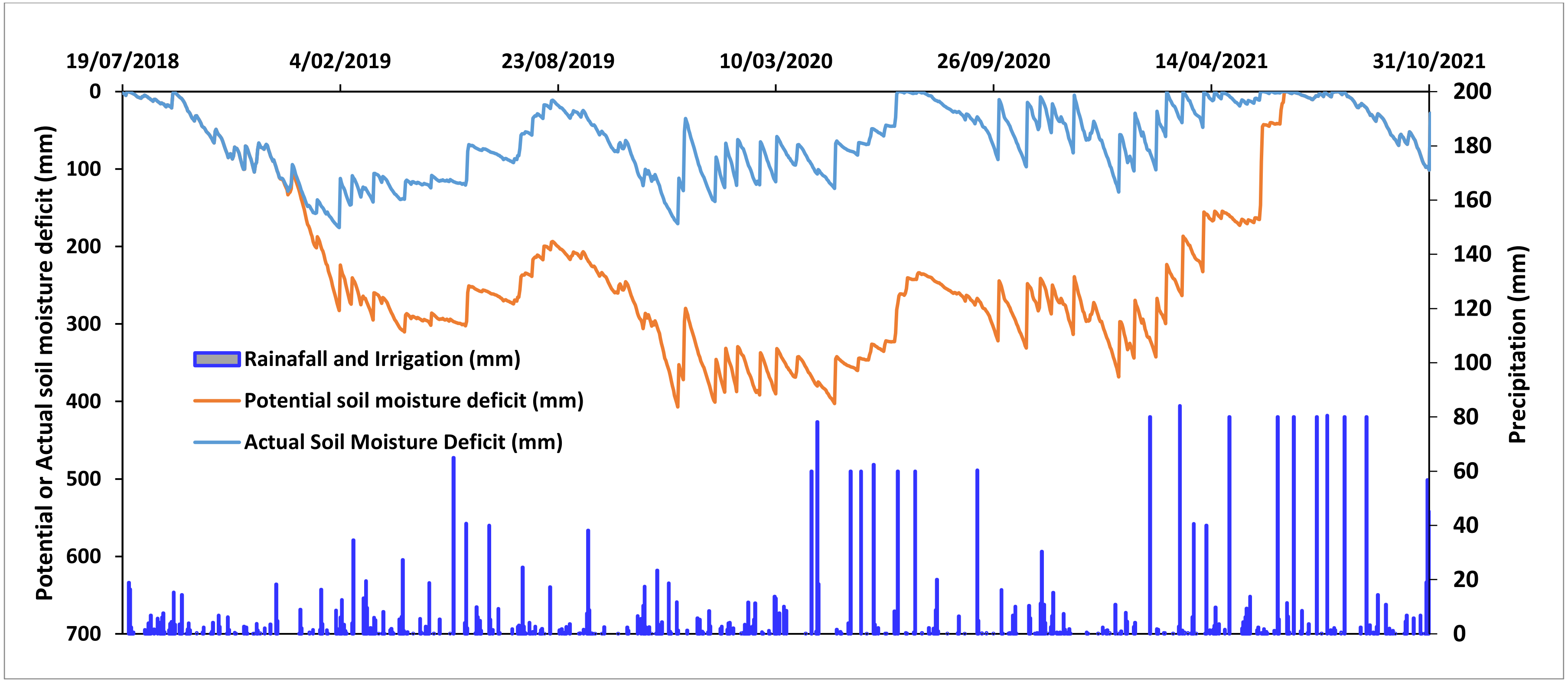


Figure 3.1 Potential soil moisture deficit (mm), actual soil moisture deficit (mm), rainfall and irrigation in a pasture diversity experiment at Lincoln University, Canterbury, New Zealand.

3.3 Experimental design

The factor of interest were the proportions of six species in the mixture (Table 3.3). Therefore, the experimental design was a six-component simplex centroid design, created in Minitab 19 statistical software. The simplex centroid had axial points and four replicates of each design point. The mixture amount was a single total of 1, which was the sum of the component proportions (X1 +X2 +X3 + X4 + X5 + X6 = 1), where X is the proportion of species, for 1 to 6 species, value ranged from $0 \leq X \leq 1$. This allowed monocultures and polyculture blends of the component species. The blends were random with each level of the replicate factor.

The simplex design identified 69 treatment mixtures. There were six monocultures, 15 binary species species mixtures (½ of each of two species), 20 three-species mixtures (⅓ of each of three species), 15 four-species mixtures (¼ of each of four species), six five-species mixtures (⅕ of each of five species), a centroid mixture (⅙ of each species) and six axial point mixtures dominated in turn by each species (7/12 of one species and 1/12 of each of the other species).

Seeds of perennial ryegrass (*Rely*) PR, cocksfoot (*Aurus*) C/CF, plantain (*Agritonic*) (P), white clover (Quartz) (WC), red clover (*Relish*) RC, and subterranean clover (*Bindoon*) SC were used to create the mixture according to simplex design. The 69 mixtures were systematically varied in the relative abundance of each species with a fixed level of overall initial abundance of 1000 seed/m^2. It is impossible to visualise the method of mixing six species in three-dimensional six-sided polygons (Hexagon). So, a typical seed mixture design for four species (PR, WC, RC and P) in a simplex format visualised as a tetrahedron is shown in Figure 3.2.

This experiment was initially designed to be a rainfall experiment. However, an external grant enables more intensive monitoring, so the experiment was irrigated. This reduces the relevance of subterranean clover. However, the changes in composition of those plots highlighted the ingress of weed species.

Table 3.2 Seeding ratio (constant overall sowing density) of 69 mixtures varying proportions of Perennial ryegrass (PR), Cocksfoot (C), Plantain (P), White clover (WC), Red clover(RC) and Sub clover (SC) in I8 experimental field at Lincoln University, Canterbury, New Zealand.

Mix	Species	RG	C0	Pl	WC	RC	SC
1	PR	1	0	0	0	0	0
2	C	0	1	0	0	0	0
3	P	0	0	1	0	0	0
4	WC	0	0	0	1	0	0
5	RC	0	0	0	0	1	0
6	SC	0	0	0	0	0	1
7	PRC	0.5	0.5	0	0	0	0
8	PRP	0.5	0	0.5	0	0	0
9	PRWC	0.5	0	0	0.5	0	0
10	PRRC	0.5	0	0	0	0.5	0
11	PRSC	0.5	0	0	0	0	0.5
12	CP	0	0.5	0.5	0	0	0
13	CWC	0	0.5	0	0.5	0	0
14	CRC	0	0.5	0	0	0.5	0
15	CSC	0	0.5	0	0	0	0.5
16	PWC	0	0	0.5	0.5	0	0
17	PRC	0	0	0.5	0	0.5	0
18	PSC	0	0	0.5	0	0	0.5
19	WCRC	0	0	0	0.5	0.5	0
20	WCSC	0	0	0	0.5	0	0.5
21	RCSC	0	0	0	0	0.5	0.5
22	PRCP	0.33	0.33	0.33	0	0	0
23	PRCWC	0.33	0.33	0	0.33	0	0
24	PRCRC	0.33	0.33	0	0	0.33	0
25	PRCSC	0.33	0.33	0	0	0	0.33
26	PRPWC	0.33	0	0.33	0.33	0	0
27	PRPRC	0.33	0	0.33	0	0.33	0
28	PRPSC	0.33	0	0.33	0	0	0.33
29	PRWCRC	0.33	0	0	0.33	0.33	0
30	PRWCSC	0.33	0	0	0.33	0	0.33
31	PRRCSC	0.33	0	0	0	0.33	0.33
32	CPWC	0	0.33	0.33	0.33	0	0
33	CPRC	0	0.33	0.33	0	0.33	0
34	CPSC	0	0.33	0.33	0	0	0.33
35	CWCRC	0	0.33	0	0.33	0.33	0
36	CWCSC	0	0.33	0	0.33	0	0.33
37	CRCSC	0	0.33	0	0	0.33	0.33
38	PWCRC	0	0	0.33	0.33	0.33	0
39	PWCSC	0	0	0.33	0.33	0	0.33
40	PRCSC	0	0	0.33	0	0.33	0.33
41	WCRCSC	0	0	0	0.33	0.33	0.33
42	PRCPWC	0.25	0.25	0.25	0.25	0	0
43	PRCPRC	0.25	0.25	0.25	0	0.25	0
44	PRCPSC	0.25	0.25	0.25	0	0	0.25

Mix	Species	RG	C0	Pl	WC	RC	SC
45	PRCWCRC	0.25	0.25	0	0.25	0.25	0
46	PRCWCSC	0.25	0.25	0	0.25	0	0.25
47	PRCRCSC	0.25	0.25	0	0	0.25	0.25
48	PRPWCRC	0.25	0	0.25	0.25	0.25	0
49	PRPWCSC	0.25	0	0.25	0.25	0	0.25
50	PRPRCSC	0.25	0	0.25	0	0.25	0.25
51	PRWCRCSC	0.25	0	0	0.25	0.25	0.25
52	CPWCRC	0	0.25	0.25	0.25	0.25	0
53	CPWCSC	0	0.25	0.25	0.25	0	0.25
54	CPRCSC	0	0.25	0.25	0	0.25	0.25
55	CWCRCSC	0	0.25	0	0.25	0.25	0.25
56	PlWCRCSC	0	0	0.25	0.25	0.25	0.25
57	PRCPWCRC	0.2	0.2	0.2	0.2	0.2	0
58	PRCPWCSC	0.2	0.2	0.2	0.2	0	0.2
59	PRCPRCSC	0.2	0.2	0.2	0	0.2	0.2
60	PRCWCRCSC	0.2	0.2	0	0.2	0.2	0.2
61	PRPWCRCSC	0.2	0	0.2	0.2	0.2	0.2
62	CPWCRCSC	0	0.2	0.2	0.2	0.2	0.2
63	PRCPWCRCSC	0.16	0.16	0.16	0.16	0.16	0.16
64	PRCPWCRCSC	0.58	0.08	0.08	0.08	0.08	0.08
65	PRCPWCRCSC	0.08	0.58	0.08	0.08	0.08	0.08
66	PRCPWCRCSC	0.08	0.08	0.58	0.08	0.08	0.08
67	PRCPWCRCSC	0.08	0.08	0.08	0.58	0.08	0.08
68	PRCPWCRCSC	0.08	0.08	0.08	0.08	0.58	0.08
69	PRCPWCRCS	0.08	0.08	0.08	0.08	0.08	0.58

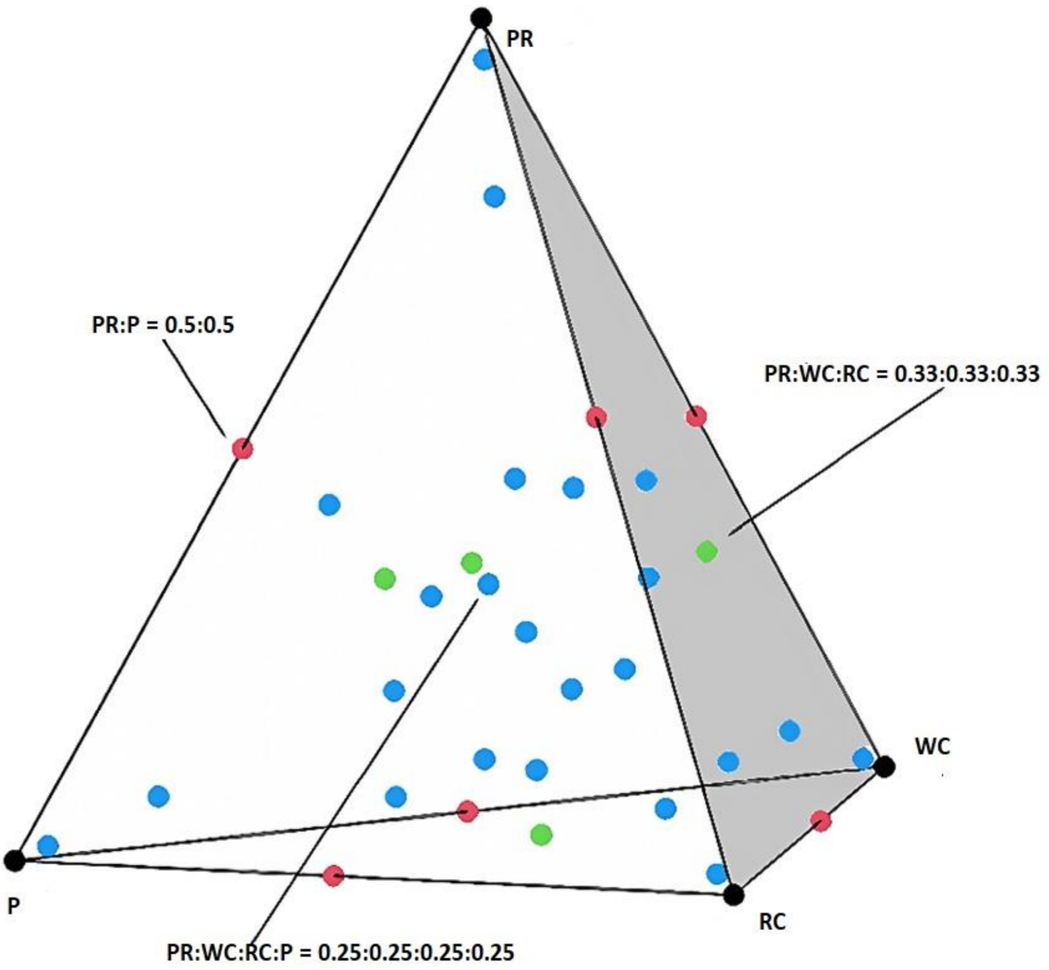


Figure 3.2 Graphical representation of typical tetrahedron design for PR, WC, RC and P four species mixture in simplex design.

Plate 3.1 Aerial photograph of the experimental field paddock in Iversen 8 at Lincoln University, Canterbury, New Zealand (Mills A, 2019)

Plate 3.2 Field experimental paddock at pre-grazing in Iversen 8 at Lincoln University, Canterbury, New Zealand.

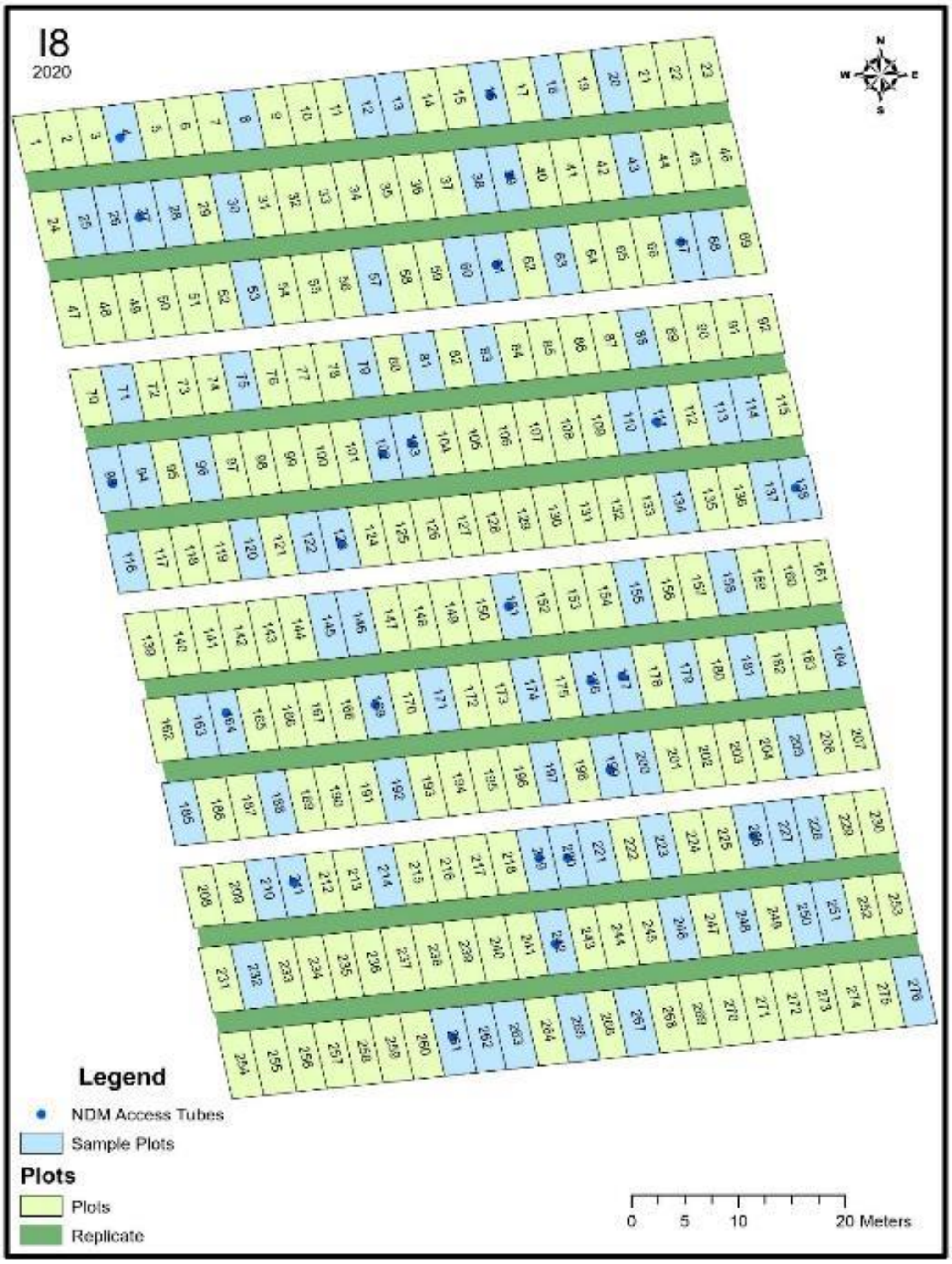


Figure 3.3 Field layout of the experiment. Replicates run North to South and black dots were monoculture plots having Neutron probe access tubes at center of each plot in Iversen 8 experimental paddock at Lincoln University, Canterbury, New Zealand.

The data of this thesis were collected over three years from 23 April 2018 (sowing) to 13 June 2021 (end of Harvest 23). Year 1 was defined as sowing to 31 May 2019 (end of Harvest 7), Year 2 as 31 May 2019 to 3 June 2020 (end of Harvest 15) and Year 3 as 3 June 2020 to 13 June 2021.

3.4 Research establishment management practices

3.4.1 **Seed germination test**

Equation 3.2 Seed germination test

A germination test was done on a random sample of seed lots of component species purchased from PGG Wrightson seeds limited, Christchurch. Four replicates of 100 seeds of each species were placed on wet blotting paper in sealed, labelled plastic containers and germinated in the incubators at a constant temperature at 20 ˚C. Distilled water was added as required to ensure moisture was non-limiting. The number of fully germinated healthy seedlings were counted 14 days after germination (ISTA, 2004). The germination percentage was an average of four replicates.

$$\text{Germination percentage (\%)} = \frac{\text{Number of total germinated seed}}{\text{The total number of seeds tested.}} \times 100$$

Thousand seed weight were 2.47, 0.92, 2.00, 0.68, 4.57 and 11.65 g, and germination percentage was (14 days after incubation at 20 ˚C) 98, 96, 93, 99, 84 and 89 for PR, C, P, WC, RC and SC, respectively.

3.4.2 **Seedbed preparation and sward establishment**

The area was sprayed on 1 March 2018 (glyphosate, 540 g/L at 2 L/ha with 200 L/ha water; WeedMaster TS540, Nufarm, NZ), cut on 12 March 2018 to remove herbage (Fieldmaster forage harvester), shallow-cultivated to 5 cm depth on 21 March 2018 to hasten the breakdown of turf (Amazone KE 2500 Special rotary harrow/wedge ring roller combination), ploughed to 20 cm depth on 13 April 2018 (Kverneland three furrow reversible plough), cultivated and reconsolidated into a seed bed on 18–19 April 2018 (rotary harrow and heavy roller), and sown to 10–15 mm depth on 23 April 2018 (air-induced plot seeder with 14 coulters spaced 150 mm apart; Flexiseeder, Christchurch, NZ).

Seed density was fixed at a single rate determined by local practice (1000 viable seeds/m^2). 1000 seed weight and germination percentage of species were obtained to achieve the constant overall sowing density. Seeds of the species were mixed according to the simplex centroid design Minitab output. These 69 mixtures, including six monocultures, were arranged in Randomised Complete Block Design (RCBD) with four replications. The plot size was 2.1 x 6 m. Twenty-three plots were arranged side by side in 12 rows, with 2 m headland between and around rows in the experimental area (Figure 3.1). The headlands were sown with perennial ryegrass ‘Rely’.

3.4.3 **Pest and weed control**

Insect pests and weeds, including plants of sown species, were not controlled. However, a non-selective herbicide (glufosinate-ammonium,200 g/L at 5 L/ha with200 L/ha water; Buster®, BASF, NZ) was sprayed on subclover monocultures on 19 March 2019 to encourage this annual to regenerate. A knapsack was used with nozzle guard to prevent droplet spread. However, only a few subclover plants emerged so it was resown by hand at grazing on 28 March 2019. Herbicide (thifensulfuron-methyl, 500 g/kg at 1.5 g/10 L water; Harmony® 50SG, DuPont, NZ) was spot-sprayed on dock (Rumex obtusifolius) plants in many plots over several days in March 2020. Subclover monocultures were again sprayed on 19 March 2020 (glufosinate-ammonium, 200 g/L at 3 L/ha with 200 L/ha water; Buster®, BASF, NZ). Selective herbicide (aminopyralid, 30 g/L at 1 L/ha with 200 L/ha water; T-Max®, Dow AgroSciences, NZ) was sprayed on plots not sown with WC or RC to control voluntary WC, on 19 March 2020. Grass grub (*Costelytra zealandica*) damaged a small area of some plots in autumn 2021, but otherwise did not warrant insecticide control, and affected areas were avoided at sampling.

3.4.4 **Grazing management**

The plots were defoliated by grazing sheep and machinery over a series of 23 harvests (Table 3.3). The number of harvests per annum was determined by local practice. At Harvests 1 and 2, the sheep were given the whole experiment. However, the sheep tended to walk up and down the experiment. This behaviour started to create hoof tracks along some of the plots. Therefore, at each harvest thereafter, the experiment was divided in half using an electric fence between Blocks 2 and 3. The sheep were given one half of the experiment at a time. The sheep were taken out of the experiment, or one half of the experiment, when a sward height of 4 cm was reached in one of the plots, which was usually after 3-7 days, depending on the herbage mass and number and type of sheep available. This was usually a legume monoculture, so overall lax grazing was used to minimise targeting these plots. The residue of the whole grazed area was then cut to 4 cm above ground level, and the clippings removed using a ride-on lawn mower, usually within 1 day of removing the sheep. If there was too much herbage for the lawn mower, a tractor and forage chopper were used followed by the lawn mower. This defoliation method helped prevent some plots from being grazed more severely than others.

Table 3.3 Defoliation details, for a pasture mix experiment at Lincoln University from 2018 to 2021.

Year Number	Harvest Number	Start Date	Finish Date	Growth Phase (d)	Defoliation Phase (d)
1	1	14/09/2018	18/09/2018	74	5
	2	19/10/2018	26/10/2018	30	8
	3	29/11/2018	07/12/2018	33	9
	4	08/01/2019	18/01/2019	29	11
	5	24/02/2019	06/03/2019	35	11
	6	28/03/2019	08/04/2019	22	12
	7	22/05/2019	31/05/2019	34	10
2	8	30/07/2019	02/08/2019	60	4
	9	13/09/2019	20/09/2019	41	8
	10	21/10/2019	29/10/2019	38	9
	11	27/11/2019	04/12/2019	28	8
	12	09/01/2020	15/01/2020	35	7
	13	18/02/2020	24/02/2020	34	7
	14	27/03/2020	02/04/2020	31	7
	15	28/05/2020	03/06/2020	56	7
3	16	28/07/2020	03/08/2020	55	7
	17	14/09/2020	21/09/2020	41	8
	18	21/10/2020	27/10/2020	31	7
	19	30/11/2020	07/12/2020	33	8
	20	12/01/2021	18/01/2021	35	7
	21	17/02/2021	22/02/2021	30	6
	22	25/03/2021	31/03/2021	31	7
	23	25/05/2021	31/05/2021	55	7

Start date was defined as the date when the sheep entered the experiment and finish date was defined as the date when the sheep exited the experiment, or, if mowing was necessary, when the experiment was mown after grazing.

3.5 Measurements

3.5.1 Herbage accumulation

For herbage biomass yield assessment, two different methods were used during the experimental period. The first method was a mechanical mower (President 6000 AL S21; Masport, NZ) for sampling pre-grazing herbage, followed by a quadrat cut for assessing post-grazing biomass. However, sheep walked up and down the mown strip, and we were concerned about the permanent damage to the residual basal sward biomass. Therefore, we used a quadrat cut sampling method after the second grazing to assess both pre and post-grazing biomass and clipped the sample at 4 and 2 cm height from ground level, respectively. In the mowing method, in the first two regrowth cycles, dry matter content was determined from a representative sample (2.1 x 6 m). The sample dry weight and fresh weight ratio were used to calculate the biomass yield. The second method involved clipping (electric clipper) herbage in a quadrat to 4 cm height, and then the field was mown to 4 cm to be uniform at the beginning of each new regrowth cycle. The residual biomass assessment was done at 2 cm from ground level. The summation of the residual biomass of monocultures to the sown proportion of species in the mixtures gave the residual biomass of mixtures. The observed residual biomass of the centroid mixture was compared with the predicted residual biomass, confirming the validity of the calculation.

3.5.2 Herbage mass assessment

Samples were stored in a cooler at 3 to 4 °C for the separation of botanical composition. For botanical composition, the sample was mixed thoroughly, divided into quarters, and diagonally opposite quarters were discarded, and the remaining quarters were mixed and repeated to get around 300 g of subsample. The subsamples were separated into the sown component species, volunteer white clover (VWC) and weeds. VWC was not separated from the sub-sample when WC was a mixture component. Separated component species were placed inside the labelled paper bag and dried with bulk samples in an oven at 65 °C for 48 hours. The dry weight of bulk and the sum of separated samples were used to calculate the biomass. The DM content of the mowing method in the first two regrowth cycles of the establishment year was assessed from the fresh and dry weight of the representative sample converted to the fresh herbage biomass of the mown area.

3.5.3 Nutritional composition

Herbage nutritional composition analysis was done on a dried bulk sample of monoculture, binary and centroid mixtures of regrowth cycles in the first three years of the experiment. The samples were milled (ultra centrifugal-mill ZM200; Retsch GmbH, Haan, Retsch-Germany) and stored in labelled plastic containers for NIRS analysis. Samples were scanned for nutritional value as Metabolizable Energy (ME) and Crude protein (CP), Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF). CP and ME content of the herbage were considered in this report, and the standard calculation procedure was described in Equation 3.3 and Equation 3.4, respectively.

Equation 3.3 Calculation of CP from N content (%)

CP = N % * 6.25

Equation 3.4 Calculation of ME content

ME= DOMD * 0.16 (Digestibility of Organic Dry Matter) (McDonald et al., 2010).

3.5.4 Meteorological measurements

3.5.5 Light interception

Sun scan canopy analyser (Delta-T Devices LTD, Burwell, Cambridge, UK) was used to measure the fraction of intercepted photosynthetically active radiation (PARi) at 7-10 day intervals. The PARi was measured in monoculture, binary and centroid mixture plots. Above (incident) and below the canopy (transmitted), PAR readings were collected from four simultaneous readings across the plot. The Sun scan sensor was placed under a canopy at 45° inclination positions across the row to avoid the row effects on readings. In each grazing cycle, the readings were obtained in full sunlight conditions (Webb et al., 2000). The sun scan readings were obtained when the canopy reached 5 – 6 cm height, enough to cover the sensor rod (1.5 cm). It took around 3 to 5 days, depending on the season. The daily fraction light interception at the beginning of the growth cycle was quantified based on proportional interpolation (linear interpolation) of NDVI values between two Greenseeker readings (Section 3.5.7). Weekly recording of NDVI values from the first day of the regrowth cycle helped to quantify the fraction of interception. Daily intercepted (PARi) was estimated from the daily fraction interception value multiplied by PAR in total incident radiation (Ro) obtained from Broadfields meteorological station. Total intercepted PAR (PARi) was calculated as a sum of daily intercepted PAR to the final date of harvest in each grazing cycle. For this calculation, weed biomass was included with the total biomass as a part of a canopy component when considering light absorption. The regression was fitted through the origin; at zero light absorption, crop/pasture has zero biomass production (Fletcher and Moot, 2007). RUE was calculated as the slope of a fitted linear relationship between DM accumulation and total absorbed PAR (Sinclair and Muchow, 1999). The fraction of light interception of forage canopies was calculated by the following equation.

Equation 3.5 Fraction light interception

$$\mathbf{FPAR_i = (PAR_{(below\ canopy)}/PAR_{(above\ canopy)}) \times 100}$$

Equation 3.6 Cumulative intercepted light energy

$$\mathbf{R_{cum} = \int_{n=1}^{n=days} R/Ro \times\ Ro\ daily}$$

R/Ro is the fraction intercepted PAR; Ro is the daily incoming PAR radiation energy.

Equation 3.7 Radiation Use Efficiency

RUE = TDM/PARi (intercepted) (Hamish et al., 2006; Rezig et al., 2010)

TDM- Total dry matter

Fraction of light interception for monoculture, binary and centroid mixtures was measured in the 2020/2021 season, except the first grazing cycle followed by winter.

3.5.6 **Soil water measurements**

Soil moisture content was continuously monitored to calculate soil moisture deficit and plan the irrigation schedule in summer and autumn to avoid swards experiencing moisture stress. Neutron probe and Time-Domain-Reflectometry (TDR) measurements were recorded at two week intervals. One access tube was placed in each of the monoculture plots permanently. The length of the aluminium tube was 2.2 m. The sensor measures soil moisture at 10 cm intervals down to the bottom of the tube. The soil moisture content in the top 20 cm was determined by Time-Domain-Reflectometry.

Plate 3.3 Topsoil moisture measurement obtained from TDR (Time Domain reflectometer) in Iversen 8 experimental site at Lincoln University, Canterbury, New Zealand.

Plate 3.4 Soil profile moisture measurement (2.2 m) by Neutron probe (CPN 580) in Iversen 8 experimental site at Lincoln University, Canterbury, New Zealand.

3.5.7 **Biological measurements**

Greenseeker measurement

Green seeker was used to determine NDVI (Normalized Difference Vegetation Index) as a green biomass ground cover (Carlson and Ripley, 1997) because the reflectance value of the sensor is highly correlated with green tissue (Chakwizira et al., 2015). The reflectance values were converted into NDVI. The readings were obtained weekly by moving the sensor in the middle of each plot by keeping the trigger pulled. The amount and colour of soil influenced the reflectance values, bare soil readings were taken after all the plots were measured and corrected NDVI values were calculated by using a scaled NDVI formula (Carlson and Ripley, 1997).

Equation 3.8 Corrected NDVI value calculation.

$$NDVI_{[corrected]} = \frac{NDVI - NDVI_o}{NDVI_s - NDVI_o}$$

Where $NDVI_o$ and $NDVI_s$ correspond to the values of NDVI for bare soils and surfaces with a fractional ground cover of ≥ 95 % full canopy cover, respectively. This reading was used to determine the green cover area before the canopy recovered to a height above the Sunscan sensor.

Platemeter reading

A rising plate meter was used to measure the compressed canopy height of species mix swards at weekly intervals. DW (Dry Weight) readings of the species mix swards were not recorded because the instrument was designed for general pasture species mix swards, especially PR and WC mix pasture. The canopy height was used to calculate the fraction of light interception at the beginning of each grazing round when the canopy was insufficient to cover the Sunscan sensor.

3.6 Statistical analysis

The procedure of quantifying the species effect as well as mixture effect as diversity contribution to dry matter yield, weed biomass, ME and CP content of herbage was described in Section 2.7.2. Three year average pasture community responses, such as biomass yield, weed biomass, crude protein and metabolisable energy content, were subjected to quadratic and cubic model analysis. A significant model was selected for further analysis and response optimization. DI model is the multiple regression method, pasture community responses are considered as the regression responses, and species proportions are the continuous predictors in the model Equation 2.5.

The persistence of pasture responses was tested based on significant interactions with higher coefficient values over the regrowth cycles. The refined significant quadratic models were subjected to response surface methodology and developed a contour plot and response surface diagram. The graphical diagram and numerical multiple response optimization accurately quantify the component proportions in the optimal seed mixture.

The regression mixture model analysis quantified the contribution of mixture component species and additional interaction contribution of species mixed swards discussed in Chapter 4. Additional statistical analyses are outlined in relevant chapters.

Chapter 4

Analysis of Pasture Attributes Averaged Across Growth Periods

4.1 Introduction

This chapter focuses on the effects of combining different proportions of pasture species in seed mixtures (as mixture ingredients) on community responses such as biomass yield and quality over time. The goal is to formulate seed mixtures that can improve pasture production over time. This research requires extensive field research work with statistical application. These objectives facilitated the main aim of the thesis were (1) to identify the species combination for improved community responses, yield, and quality, (2) to quantify the diversity contribution of considered community responses, (3) to assess the stability of those responses over time based on interspecific interaction contribution, and (4) to identify the optimal component proportion for improved yield and quality attributes. These objectives answer several questions: How many species and which species in the mixture combination and their proportion improved the yield and quality over time? The result section of this chapter first explains the DI model output for the primary pasture response, DM yield.

4.2 Materials and Methods

The quantifying of the pasture responses in the model approach and the procedure of measuring yield and quality are described in Section 2.7.2 and 3.5.

4.3 Results

4.3.1 **Observed pasture attributes**

Observed pasture yields and quality attributes are summarised as averages across blocks and growth periods. The average value of the four considered pasture responses is given in Table 4.11 (end of this chapter). The overall average yield of species mixed swards per regrowth cycle was 1.70 t DM/ha (13.6 t DM/ha per year), average weed was 0.23 t DM/ha (13.5% of yield), average ME and CP were 10.8 MJ/kg DM and 20.7%, respectively. There was variation in each response, with several seed mixtures across the sward mixture communities giving above-average yield, weed suppression, ME, and CP content. Among the 34 identified best entries, the seed mixture of 1/5 perennial ryegrass, plantain, white clover, red clover, and subterranean clover produced the greatest observed yield of 2.05 t DM/ha (16.4 t DM/ha per year) with a lower weed content of 0.09 t DM/ha (4.4%) per regrowth cycle. In the binary mixture combinations, the highest ME of 11.2 MJ/kg DM was in the PR*SC mixture, and the highest CP of 25.0% was in WC*SC. The response optimisation (Section 4.4.5) considered the four responses simultaneously and optimized the seed mixture proportion for improving herbage biomass yield and quality. PR*WC and PR*RC binary mixtures gave a biomass yield of 1.70 (13.6 t/ha/yr) and 1.90 t/ha (15.2 t/ha/yr) and a lower weed biomass yield of 0.09 and 0.19 t/ha, respectively. The weed biomass yield of the binary mixtures was lower than the monoculture average. The species mixed swards produced 16.5% higher biomass yield and 64% lower weed biomass than the monoculture average.

4.3.2 **Botanical composition**

Pasture composition as a percentage of sown species botanical composition in dry weight differed between monocultures and mixtures. Table 4.12 and Table 4.13 (end of Chapter) show the swards' average biomass composition. Sown biomass yield of monoculture species differed among species. On average, mixtures gave a higher proportion of sown species (0.86) than the monoculture average (0.56). The sown proportion of species fluctuated in mixture communities depending on their functional groups and the number of species in the mixtures. Increasing the number of species in the mixture reduced the unsown species proportion (Table 4.13). The botanical composition proportion of grasses in monoculture was increased from the establishment cycle year to the 3rd year, both in perennial ryegrass and cocksfoot. The sown proportion of plantain continuously reduced, with a sharp decline from the 2nd year to the 3rd year. The highest proportion of unsown species was in the clover monocultures with ranges from 0.40 in red clover to 0.73 in sub-clover. On average, the white clover had 0.53 unsown species over three years (Table 4.13).

The sown proportion of the sub-clover reduced rapidly after the establishment year and ended up with a greater fraction of unsown species in the 2nd and 3rd years. The sown and unsown species composition dynamics of monocultures and mixtures PR*WC and PR*RC are presented in Table 4.13. The sown proportion of PR*WC and PR*RC binary mixtures ranged from 0.88 to 0.96 over three years. Binary and ternary mixtures had three-year average sown proportions of 0.74 and 0.84, respectively. A detailed analysis of the composition shifting pattern is described in Chapter 5.

4.3.3 **Regressions of average pasture attributes versus species proportions of seed mixture**

Table 4.1 and Table 4.5 show estimated coefficient values from three years' average pasture DM yield. The Diversity-Interaction (DI) model uses species proportion and interaction as predictors in the regression framework. Output revealed that the differences in the above-ground biomass yield of mixtures were based on the associated component species proportions. The coefficient value of monoculture represented the potential/modelled yield of the monoculture species, which differed ($p<0.01$) among species. The model fit was denoted by the P value of the linear component in the quadratic model. The modelled yield of the monocultures ranged from 1.19 t/ha (9.5 t/ha) in cocksfoot to 1.64 t/ha (13 t/ha) in red clover. The model output did not have P or ‘t’ values in the coefficient table because of no associated component species or interactions.

In binary components, the P value represents the significant interaction between component species. The coefficient value of quadratic or cubic models revealed the magnitude and direction of the mixture effect between the component species (Table 4.1 and Table 4.5). Modelling of three-year average biomass yield showed strong positive and negative diversity effects. The nine binary mixture combinations that showed significant interactions are highlighted in Table 4.4. The positive interaction coefficient term indicated that the component species in the mixture interacted synergistically. When the model response value for DM production was greater than expected from proportional monoculture performance it was categorized as “outyielding mixture”. The negative coefficient value for the considered response indicates the component species act antagonistically as was observed for weed biomass yield. The mixture performance would be higher (higher interaction contribution for weed suppression) than the expected proportional performance of the monoculture in the mixture, and a strong effect was observed for weed biomass yield.

The standard error (SE coef) of the coefficient value (Table 4.1) represents how precisely the model estimated the coefficient values. The values were obtained from mean square errors divided by the number of observations. For example, for monoculture and binary mixtures, the values were 0.08 and 0.34, respectively. They were standard deviations of the estimates. The more precise the estimate, the lower the SE value. The SE for each estimated regression coefficient for six monoculture species and 15 binary mixtures were similar within the group in the simplex design matrix.

Table 4.1 Quadratic regression analysis for three years overall mean DM yield data against the initial sown species proportion of six species (Minitab output) in a diverse pasture species mixture experiment at Lincoln University, Canterbury, New Zealand.

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.1258	0.0197	6.39	0.000	1.50
2	0.0200	0.0197	1.02	0.310	1.50
3	-0.140	0.0197	-7.13	0.000	1.50
PR	1.5222	0.0889	*	*	4.07
C	1.1887	0.0889	*	*	4.07
P	1.5006	0.0889	*	*	4.07
WC	1.3625	0.0889	*	*	4.07
RC	1.6358	0.0889	*	*	4.07
SC	1.3328	0.0889	*	*	4.07
PR*C	0.741	0.342	2.17	0.031	1.94
PR*P	0.830	0.342	2.43	0.016	1.94
PR*WC	1.193	0.342	3.49	0.001	1.94
PR*RC	1.642	0.342	4.80	0.000	1.94
PR*SC	1.385	0.342	4.05	0.000	1.94
C*P	0.765	0.342	2.24	0.026	1.94
C*WC	0.936	0.342	2.74	0.007	1.94
C*RC	2.046	0.342	5.98	0.000	1.94
C*SC	0.387	0.342	1.13	0.259	1.94
P*WC	0.544	0.342	1.59	0.113	1.94
P*RC	0.182	0.342	0.53	0.595	1.94
P*SC	0.258	0.342	0.75	0.451	1.94
WC*RC	0.793	0.342	2.32	0.021	1.94
WC*SC	0.657	0.342	1.92	0.056	1.94
RC*SC	0.525	0.342	1.53	0.126	1.94

PR- perennial ryegrass; C-cocksfoot; P-plantain; WC- white clover; RC- Red clover; SC-sub clover.

The coefficient value divided by the standard error represented the T value shown in Table 4.1. The p-value associated with T statistics is less than the standard significant level ($P<0.05$ or 0.01), representing the significant interaction value. In repeated grazing/sampling, evaluating the P value for significant interaction between co-occurring species in the mixture combination with associated higher coefficient values over time is one of the criteria for selecting the response stability of the respective mixture.

VIF in Table 4.1 is the Variance Inflation Factor indices. It expresses how the variance of the calculated coefficient values was inflated in the model's prediction (MINITAB 20). VIF value is categorized into three levels, < 1, 1-5 and > 5, which indicates no correlation, moderate correlation, and high correlation between the predictor and response.

Table 4.2 The model summary of the quadratic regression analysis for three years' average DM in diverse pasture species mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.19	49.7%	45%	10.6	40.5%

The model summary is given in Table 4.2. where S value 0.19 represents the standard deviation of the distance between the observed (measured data) values and fitted values. The unit for S was (t/ha) similar to the unit of the considered response. The PRESS (Predicted Error Sums of Square) value indicated the deviation between the fitted and observed values. This was the sum of the square of the residual error (SEE) or prediction residual for the observation (Allen, 1974; Tarpey, 2000). This is used to cross-validate the regression matrix. The lower value of 10 represents the predictability of the model for herbage biomass yield and was considered a better model.

The significant model had a moderate fit to the data (R^2 = 49.7%, R^2adj = 45%). R-sq in the model summary table represents the percentage of variation in the response (dependent variable-herbage biomass) explained by the independent variable in the model. The model value can range from 0 to 100%. This value explains how well the model fits the collected data. The higher the value, the better the model fits the data, and the model is well explained to the response. The quadratic model R^2 value for DM yield was 49.7%, showing that 50% of data had a reasonable fit to the model (Table 4.2).

Table 4.3 Analysis of variance table associated with the quadratic model analysis based on three years average biomass yield of a diverse pasture species mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Blocks	3	2.4807	2.48074	0.82691	23.20	0.000
Regression	20	6.3911	6.39113	0.31956	8.96	0.000
Linear	5	2.9948	0.56522	0.11304	3.17	0.009
Quadratic	15	3.3963	3.39630	0.22642	6.35	0.000
PR*C	1	0.0173	0.16724	0.16724	4.69	0.031
PR*P	1	0.0956	0.20983	0.20983	5.89	0.016
PR*WC	1	0.2416	0.43371	0.43371	12.17	0.001
PR*RC	1	0.5910	0.82170	0.82170	23.05	0.000
PR*SC	1	0.5764	0.58409	0.58409	16.39	0.000
C*P	1	0.0901	0.17826	0.17826	5.00	0.026
C*WC	1	0.1367	0.26706	0.26706	7.49	0.007
C*RC	1	1.1860	1.27581	1.27581	35.79	0.000
C*SC	1	0.0321	0.04568	0.04568	1.28	0.259
P*WC	1	0.0587	0.09029	0.09029	2.53	0.113
P*RC	1	0.0026	0.01011	0.01011	0.28	0.595
P*SC	1	0.0116	0.02027	0.02027	0.57	0.451
WC*RC	1	0.1556	0.19156	0.19156	5.37	0.021
WC*SC	1	0.1170	0.13156	0.13156	3.69	0.056
RC*SC	1	0.0839	0.08392	0.08392	2.35	0.126
Residual Error	252	8.9830	8.98295	0.03565		
Total	275	17.8548				

PR- perennial ryegrass; C-cocksfoot; P-plantain; WC- white clover; RC- Red clover; SC-sub clover.

The ANOVA table expressed the model's significance, having different P values from lower to higher models, linear to quadratic or cubic models. The model P-value in ANOVA represents the level of significant association between the biomass yield and the component proportion. A significant linear model indicates significant herbage biomass yield differences among the monoculture species ($P < 0.01$). The significant quadratic model over the linear model represents that the DM production of mixtures (binary) differed among them with a number of interactions between component species ($P < 0.01$). The ANOVA table included the associated species terms in a respective order with F and P-values. The lower P-value of the term ($p<0.05$) indicates the interaction association between component species.

4.3.4 **Prediction of herbage biomass yield**

Quadratic model analysis output associated with ANOVA showed that the linear and quadratic components of the regression were significant ($P<0.001$) for above-ground biomass. The biomass yield of monoculture species differed ($P<0.05$), and their proportion in binary mixtures also differed among species ($p<0.05$). The coefficient value of binary mixtures expresses the interaction between co-occurring species. The quadratic model identified nine mixture combinations for higher estimated yield with significant interactions between component species, highlighted in Table 4.4. These mixture communities produced higher yields (P<0.01) than monocultures. The modelled average yield across the binary mixture combinations was 0.21 t/ha (1.7 t/ha/yr). The positive coefficient values ranged from 0.26 to 2.05, showing the magnitude of the diversity effect. The highest beneficial diversity biomass productivity of 0.51 t/ha (4.1 t/ha/yr) was recorded in C*RC and had the highest interaction coefficient value of 2.05. The binary mixtures such as PR*WC, PR*RC, PR*SC and C*RC showed a significant interaction with high interaction coefficient values, indicating the potential of mixture performance. The beneficial DM production value ranged from 0.04 (0.32/t/ha/yr) in P*RC to 0.51 t/ha (4 t/ha/yr) in C*RC. The modelled coefficient value, modelled yield benefit and actual yield are presented in Table 4.4. The maximum modelled yield was in PR*RC, which produced a 1.98 t/ha (15 t/ha) DM yield, higher than the PR*WC mixture, which gave the biomass yield of 1.73 t/ha (13.3 t/ha). In addition to that, the interaction between the associated species in C*RC with higher interaction coefficient value improved the herbage biomass yield.

4.4 Optimisation of mixture components

The significant quadratic and cubic model explained the effect of component species proportion on community response/s and was used for optimisation. The response surface investigation through a significant model quantified the beneficial diversity contribution, identified the process variable (species proportion) for maximising the component proportion for individual responses, and simultaneously considered all four responses (optimiser) to achieve the goal of identifying productive pasture species mixture proportions. Several responses (biomass yield, weed yield, ME and CP) were simultaneously considered in Minitab (version 20) optimisation, and the interactive (plot) (Figure 4.9; Figure 4.10 and Figure 4.11) allows us to adjust the component species proportions to maximise the multiple responses.

Table 4.4. The coefficient values from the quadratic model, modelled yield, actual yield, and diversity contribution from 2018 to 2021 in a diverse pasture species mixture experiment under irrigated conditions at Lincoln University, Canterbury, New Zealand.

Mixture	Coefficient	Modelled yield (t/ha)	Actual yield (t/ha)	Modelled Diversity yield (t/ha)
PR	1.52	1.52	1.53	
C	1.18	1.18	1.21	
P	1.50	1.50	1.52	
WC	1.36	1.36	1.41	
RC	1.63	1.63	1.67	
SC	1.33	1.33	1.38	
PR-C	0.74	1.53	1.54	0.18
PR-P	0.83	1.72	1.72	0.21
PR-WC	1.19	1.73	1.70	0.29
PR-RC	1.64	1.98	1.90	0.41
PR-SC	1.38	1.76	1.71	0.34
C-P	0.76	1.53	1.54	0.19
C-WC	0.94	1.50	1.50	0.23
C-RC	2.05	1.91	1.94	0.51
C-SC	0.39	1.35	1.31	0.10
P-WC	0.54	1.56	1.51	0.13
P-RC	0.18	1.60	1.55	0.04
P-SC	0.26	1.47	1.41	0.06
WC-RC	0.79	1.68	1.62	0.19
WC-SC	0.66	1.50	1.41	0.16
RC-SC	0.52	1.61	1.56	0.13

The highlighted components showed significant interaction at p <=0.05. Quadratic model is significant at P= 0.01. R^2=49%, R^2(adj)=45%.

4.4.1 Quantifying diversity-biomass productivity of ternary mixtures (cubic model)

The cubic model refinement process was carried out and is presented in Figure 4.5. The cubic model analysis for DM production was not statistically significant when including all terms in the model (Appendix A). The re-analysis of the (refined) model after removing non-significant terms improved the model analysis and produced a significant (P<0.01) model. The improved model showed the additional benefit of adding a third species into the mixture (Table 4.6). The refined cubic model summary revealed that data had a considerable fit (R^2= 50.4%) (Appendix A). The modelled yield of PR and RC in the monocultures were 1.66 t/ha (13 t/ha/yr) and 1.70 t/ha (14 t/ha/yr), respectively. WC showed a potential yield of 1.44 t/ha (11.5 t/ha/yr). Based on the refined model, PR, had a significant interaction with all other species combinations in this experiment, and C had a significant interaction only with RC and WC. Mixtures showing a significant interaction between the components are highlighted in Table 4.5.

Table 4.5 Refined cubic model analysis for three years of average DM yield data (2018 to 2021) from a multi-species mixture experiment at Lincoln University, Canterbury, New Zealand.

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.1258	0.0194	6.49	0.000	1.50
2	0.0200	0.0194	1.03	0.303	1.50
3	-0.140	0.0194	-7.24	0.000	1.50
PR	1.6568	0.0743	*	*	2.93
C	1.3054	0.0656	*	*	2.29
P	1.5952	0.0534	*	*	1.52
WC	1.4404	0.0671	*	*	2.39
RC	1.6998	0.0661	*	*	2.32
SC	1.4251	0.0589	*	*	1.84
PR*WC	0.664	0.377	1.76	0.080	2.42
PR*RC	1.069	0.377	2.84	0.005	2.42
PR*SC	0.766	0.376	2.03	0.043	2.42
C*WC	0.856	0.333	2.57	0.011	1.89
C*RC	1.962	0.333	5.90	0.000	1.89
PR*C*P	6.08	2.16	2.82	0.005	1.27
PR*WC*RC	4.14	2.42	1.71	0.088	1.60
PR*WC*SC	4.55	2.42	1.88	0.061	1.60
PR*RC*SC	5.27	2.41	2.19	0.029	1.59
P*WC*RC	5.20	2.17	2.39	0.017	1.29
P*WC*SC	4.74	2.17	2.19	0.030	1.29

The cubic modelled yield, beneficial diversity biomass productivity and actual yield are given in Table 4.6. The refined model improved the yield estimation and optimised (Figure 4.1) the component species for maximising DM production. The refined cubic model's linear component was significant (<0.01), which showed that the DM production of monocultures was different among them. The component species proportion influenced biomass production ($p<0.05$) in ternary mixtures (Appendix A). The refined cubic model showed slight changes in coefficient values, representing the significant species effect on estimated DM yield (Appendix B). The interaction coefficient values of the binary mixtures were positive and ranged from 0.66 in PR*WC to 1.96 in C*RC, giving diversity an attributed beneficial biomass yield of 0.16 t/ha (1.28 t/ha/yr) and 0.46 t/ha (2.16 t/ha/yr), respectively. Across the mixtures, ternary mixtures gave an additional average biomass yield ranging from 0.11 to 0.16 t/ha, which was lower (<1.3 t/ha/yr) than the average of binary mixtures. PR*RC produced 0.27 t/ha (2.16 t/ha/yr) over the experimental duration. The model analysis did not consider the fourth species in the mixtures, this means the inclusion of the fourth species did not significantly improve the yield except for the identity contribution (monoculture) towards improving DM yield.

Table 4.6 Quantifying the interaction turnover, modelled yield from cubic model and observed yield in a multi-species mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Mixture	Modelled yield (t/ha)	Actual yield (t/ha)	Interaction turnover (t/ha)
PR	1.65	1.53	
Co	1.30	1.21	
Pl	1.59	1.52	
WC	1.44	1.41	
RC	1.69	1.67	
SC	1.42	1.38	
PR*WC	1.54	1.70	0.16
PR*RC	1.66	1.90	0.27
PR*SC	1.53	1.71	0.19
C*WC	1.37	1.50	0.21
C*RC	1.49	1.94	0.46
PR*C*P	1.50	1.63	0.16
PR*WC*RC	1.58	1.86	0.11
PR*WC*SC	1.49	1.81	0.12
PR*RC*SC	1.57	1.88	0.14
P*WC*RC	1.55	1.68	0.14
P*WC*SC	1.47	1.59	0.13

The refined cubic model analysis showed that the PR*WC*RC mixtures showed an interaction between the component species at p=0.08 for PR*WC and PR*WC*RC mixtures. The quadratic model analysis for DM yield of individual regrowth cycles showed significant interactions between the component species of the binary mixtures in PR*WC and PR*RC occurred 7 and 9 times, randomly occurring over 23 re-growth cycles (Appendix I). These binary mixtures had synergistic interactions and higher coefficient values over time, indicating improved production. The sum of pairwise interaction when combining PR with WC and RC in above binary mixtures was the main contributor to the mixture yield response. The estimated yield of PR*WC*RC in the cubic model produced an additional DM yield of 2.6 t/ha during the experimental period (P=0.08). The estimated DM yield per regrowth cycle of the above mixture was 1.86 ± 0.21 t/ha (15 t/ha/yr), and was identified as a "transgressive overyielding", higher than the best monoculture RC (1.67 t/ha and 13 t/ha/yr). The contour plot and response surface diagram showed the optimal component species for higher DM yield. The darkest circle close to the PR*RC edge gave the highest yield of > 1.9 t/ha. This mixture comprised a higher proportion of PR/RC and a lesser proportion of WC in the optimal mixture. The numerical optimisation accurately quantified the proportion of these species while simultaneously considering the multiple responses (Figure 4.9, 4.10 and 4.11).

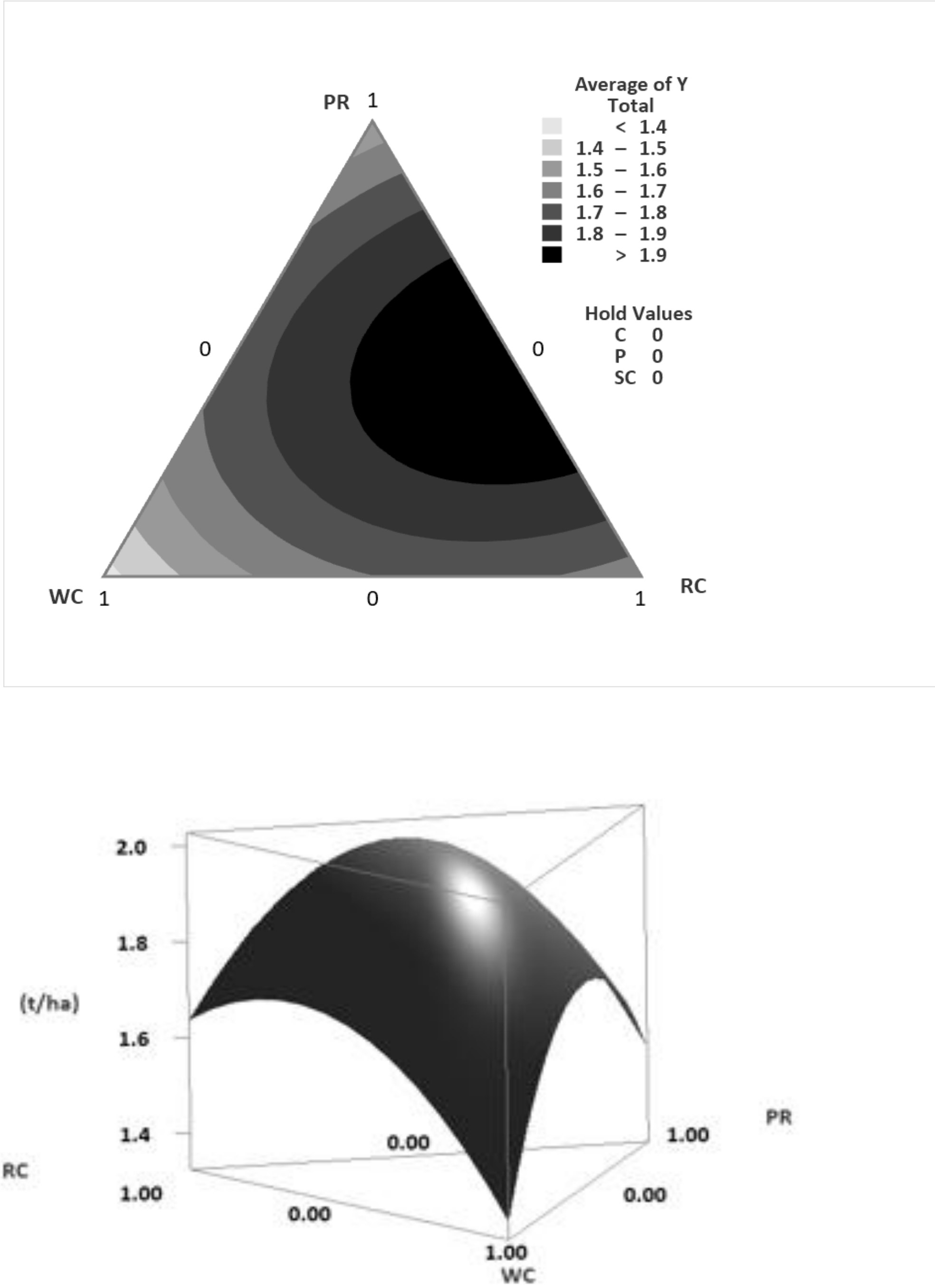


Figure 4.1 Ternary contour plot and response surface diagram from a quadratic model fitted to the data over three years average plot yield as a function of sown proportions of PR, WC and RC from 2018 to 2021 in a multi-species mixture experiment at Lincoln University, Canterbury, New Zealand.

4.4.2 Identity and diversity effects on weed suppression

The overall performance of pasture swards related to weed suppression showed that the mixtures effectively suppressed the weed growth and produced a lower weed biomass yield (0.23 t/ha) than their monoculture average (0.64 t/ha). The weed suppressive effect of diverse species mixed pasture swards was strong and increased with the increasing number of species in the mixtures. The significant linear model indicated that monoculture species differed in their ability to suppress weed biomass (P=0.05). Among the monocultures, the modelled weed biomass yield ranged from 0.23 t/ha in PR to 1.12 t/ha in SC. The modelled weed biomass yield of C, WC, P and RC were 0.33, 0.73, 0.75 and 0.63 t/ha, respectively. The modelled weed biomass of monoculture species was less than 1 t/ha, except for sub-clover (1.12 t/ha). The DI modelling approach identified that species proportion in the mixture showed a strong diversity effect on weed biomass. The quadratic and cubic models were significant ($P < 0.01$), indicating that weed suppression or biomass yield was lower even in binary or ternary mixtures. The significant cubic model analysis is given in Equation 4.1, and significant interactions between the component species in the mixtures are highlighted in the equation (Appendix C).

Equation 4.1 Cubic model analysis for weed biomass (DMY-W)

DMY-W = 0.23 PR + 0.33 C + 0.75 P + 0.73 WC + 0.63 RC + 1.12 SC - **0.98 PRP** -**1.59 PRWC** -**1.09 PRRC** - **1.95 PRSC** - **0.86 CP** - **1.36 CWC** - **0.83 CRC** - **1.83 CSC** - **1.69 PWC** - **1.12 PRC** - **1.33 PSC** - **1.22 WCSC** -**0.65 RCSC** - **4.70 PRWCRC** -**2.78 PRWCSC** – **3.38 PRWCRC** – **3.25 PRCSC** – **4.25 WCRCSC** (R^2=79.2%, R^2(adj)=77.0%, P=0.001)

In the special cubic model, the significant (p=0.00) linear, quadratic and cubic model revealed that the modelled weed biomass yield of the monoculture, binary and ternary mixtures differed ($P<0.05$) and strongly suppressed weed growth, denoted by the negative coefficient value less. The significant negative coefficient value of mixtures implied that they had a greater influence on weed suppression and reduced the weed biomass yield more than their relative proportion of monoculture weed suppression. The model analysis for average weed biomass of mixtures over three years revealed that 33% of the mixture combinations showed transgressive weed suppression. These mixture combinations produced lower weed biomass yield than the lowest weed biomass yielding monoculture component species PR. Transgressive weed suppression persisted in several mixtures. Among the mixture components, more priority was given to binary mixtures such as PR*WC, PR*RC and the three species mixture PR*WC*RC to optimise economically important responses over the several re-growth cycles with higher coefficient values in the quadratic model. The modelled weed biomass yield of PR*WC, PR*RC, PR*P, C*P, C*WC, C*RC and PR*WC*RC were 0.09, 0.16, 0.25, 0.33, 0.19, 0.28 and 0.34 t/ha, respectively (Table 4.7).

The ternary contour plot and response surface diagram developed from the significant cubic model which expressed the optimal component species proportion for minimal weed biomass yield. The light-coloured region above the centre of the plot shows that the higher proportion of PR in association with WC and RC strongly suppressed weed and produced lower weed biomass yield. The response surface diagram searched for lower biomass regions in a three-dimensional plane and showed the optimised species proportions to achieve the lowest weed biomass (< 0.05 t/ha) or minimise weed growth. The light-coloured region in the middle of the triangle, close to PR, as shown in Figure 4.2, produced a weed biomass yield of 0 – 0.15 t/ha. The response optimizer in Figures 4.9, 4.10 and 4.11 minimise weed biomass at various proportional settings such as PR:WC = 50:50, PR:WC:RC = 45:11:44, PR:WC:RC = 50:15:35, as discussed in Section 4.4.5.

Table 4.7 Cubic model analysis output for quantifying weed biomass yield of ternary mixtures from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Mixture	Modelled coefficient value	Actual yield (t/ha)	Modelled weed yield (t/ha- equal to sown monoculture proportion)	Diversity attributed to lower weed biomass (t/ha)	Modelled weed biomass yield of mixtures (t/ha)
PR	0.23	0.20			
C	0.33	0.30			
P	0.75	0.76			
WC	0.73	0.73			
RC	0.63	0.65			
SC	1.12	1.17			
PR*P	- 0.98	0.25	0.49	- 0.24	0.25
PR*WC	- 1.59	0.09	0.48	- 0.39	0.09
PR*RC	-1.09	0.19	0.43	- 0.27	0.16
PR*SC	- 1.95	0.17	0.67	- 0.48	0.19
C*P	- 0.86	0.34	0.54	- 0.21	0.33
C*WC	- 1.36	0.27	0.53	- 0.34	0.19
C*RC	- 0.83	0.34	0.48	- 0.20	0.28
C*SC	- 1.83	0.29	0.72	- 0.45	0.27
P*WC	- 1.69	0.41	0.74	- 0.42	0.32
P*RC	- 1.12	0.47	0.64	- 0.28	0.36
P*SC	- 1.33	0.65	0.93	- 0.33	0.60
WC*SC	- 1.22	0.67	0.92	- 0.30	0.62
RC*SC	- 0.65	0.76	0.87	- 0.16	0.71
PR*WC*RC	- 4.70	0.09	0.51	- 0.17	0.34
PR*WC*SC	-2.78	0.13	0.68	- 0.01	0.67
P*WC*RC	- 3.38	0.28	0.68	- 0.12	0.56
P*RC*SC	- 3.25	0.38	0.82	- 0.11	0.71
WC*RC*SC	- 4.25	0.50	0.81	- 0.15	0.66

PR-based mixtures strongly suppressed weed growth and produced lower weed biomass than cocksfoot-based pasture mixtures. The lower modelled biomass yield of 0.09 and 0.16 (0.72 to 1.3 t/ha/yr) was in PR*WC and PR*RC combinations. The weed biomass yield of the C*WC was 0.19 t/ha. The modelled weed biomass of 0.56 t/ha was obtained in the PR*WC*RC ternary mixture at the ratio of 0.33 of each mixture component (number of plants), which produced lower weed biomass (Table 4.7). As expected mixtures containing sub-clover as a component species allowed more room for the growth of unsown species (winnter annuals) and produced higher weed biomass yield than the other mixtures.

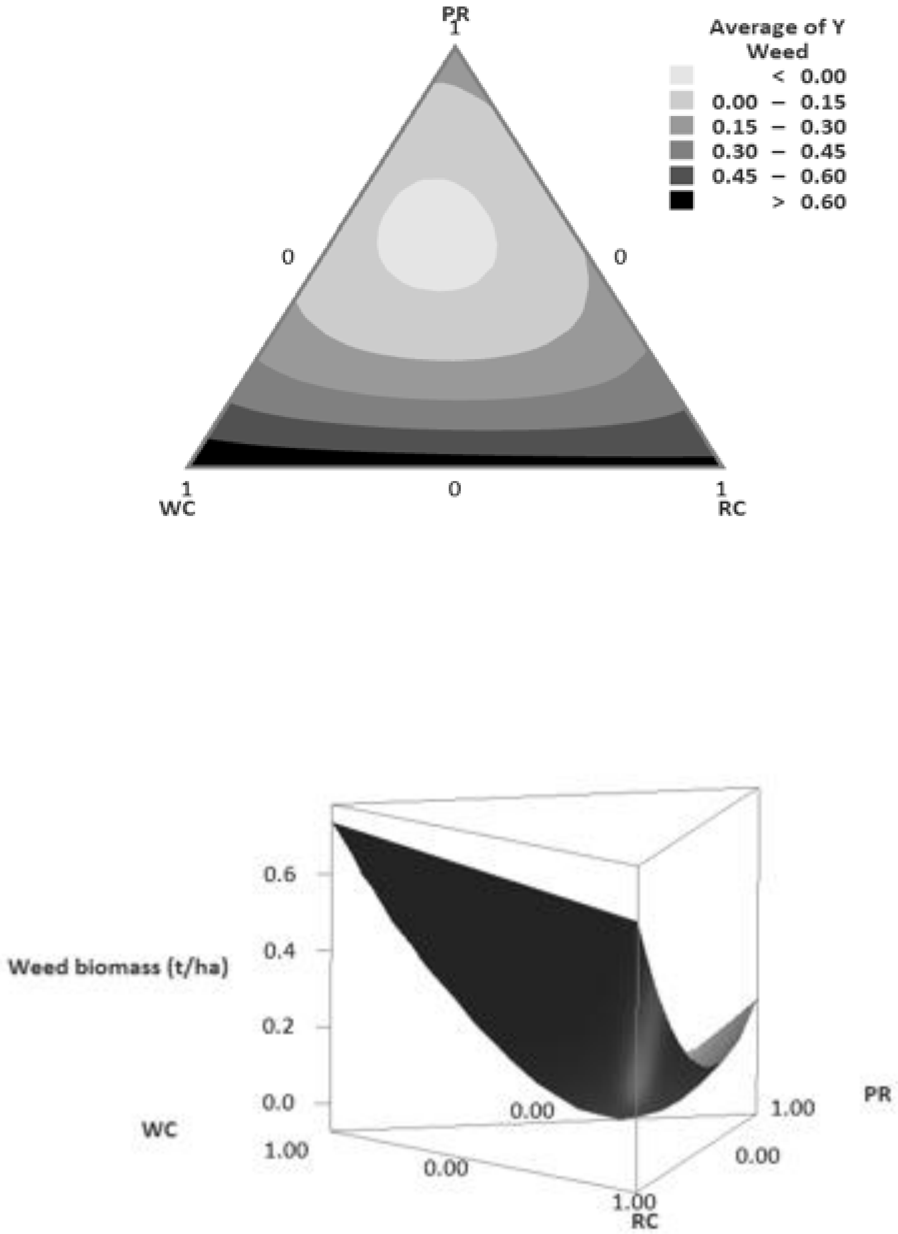


Figure 4.2 Ternary contour plot and response surface diagram of weed biomass fitted from a cubic model analysis in a diverse species mixed pasture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

4.4.3 **Nutritional composition of swards**

The three-year mean ME and CP content of herbage biomass of monocultures, binary and centroid mixtures are presented in Table 4.8. The ME content of monoculture swards ranged from 10.5 to 11.2 MJ ME kg^{-1} in RC and PR, respectively. The CP content ranged from 16.8% in PR to 24.3% in RC. Among the binary mixtures, the lowest and highest CP content of 16.3% and 24.9% were recorded in PR*C and WC*SC mixtures, respectively. The ME and CP content of the centroid mixture was 10.9 MJ ME kg^{-1} and 20.6%. The legume functional component in the mixture improved the CP content of herbage materials.

Table 4.8 The average ME and CP content of monocultures and mixtures in a multi-species pasture mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

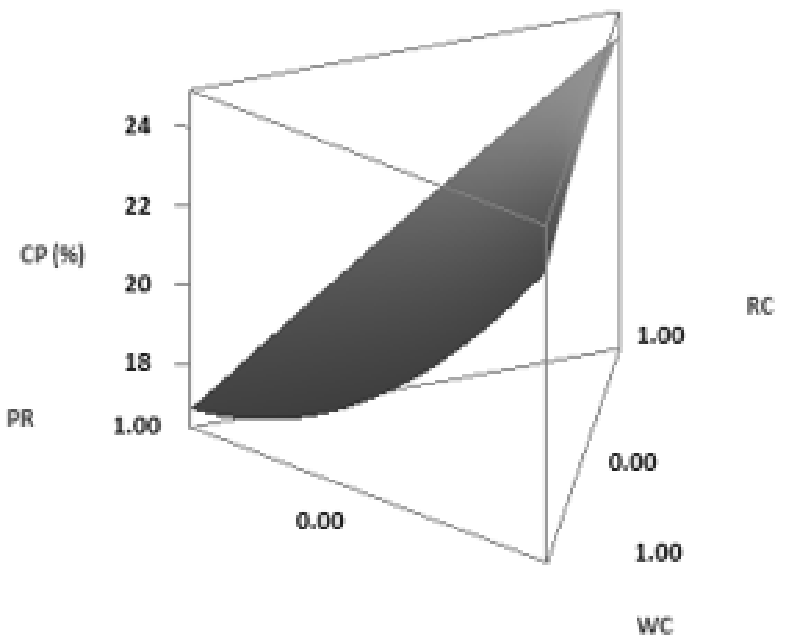
Sward	X1:PR	X2:C	X3:P	X4: WC	X5:RC	X6:SC	ME (MJ ME/kg DM)	CP (%)
1	1	0	0	0	0	0	11.0	16.9
2	0	1	0	0	0	0	10.7	18.6
3	0	0	1	0	0	0	10.9	21.7
4	0	0	0	1	0	0	10.7	23.7
5	0	0	0	0	1	0	10.5	24.3
6	0	0	0	0	0	1	10.8	20.7
7	1/2	1/2	0	0	0	0	11.0	16.3
8	1/2	0	1/2	0	0	0	11.0	17.7
9	1/2	0	00	1/2	0	0	11.1	18.8
10	1/2	0	0	0	1/2	0	10.8	20.7
11	1/2	0	0	0	0	1/2	11.2	16.7
12	0	1/2	1/2	0	0	0	10.8	19.1
13	0	1/2	0	0	1/2	0	10.8	20.2
14	0	1/2	0	0	1/2	0	10.6	22.0
15	0	1/2	0	0	0	1/2	10.8	19.1
16	0	0	1/2	1/2	0	0	10.9	21.0
17	0	0	1/2	0	1/2	0	10.7	22.7
18	0	0	1/2	0	0	1/2	10.8	21.6
19	0	0	0	1/2	1/2	0	10.7	24.4
20	0	0	0	1/2	0	1/2	11.0	25.0
21	0	0	0	0	1/2	1/2	10.7	24.8
63	1/6	1/6	1/6	1/6	1/6	1/6	10.8	20.7

Diversity effect on the crude protein content of swards

The three-year average CP content of herbage materials was modelled to the initial sown proportions, which showed that the species proportion ($P < 0.01$) influenced the protein content of herbage materials. The DI model table indicated that 95.4% of the variance in data fit with the model. The monoculture species were ($P<0.01$) different. The modelled co-efficient values for RC, WC, P, SC, C and PR were 24.3, 23.7, 21.6, 20.7, 18.6 and 16.8%, respectively. The interaction coefficient values were positive and negative, as shown in Equation 4.2. The lower coefficient value (<1) indicated that binary combinations did not improve the protein content of herbage material. The CP content of the species mixed pasture swards was lower than what would be predicted based on the proportional contribution of the component monocultures. The significant interaction between the component species in the binary mixture, such as WC*RC and RC*SC, is highlighted in Equation 4.2. The higher positive interaction coefficient values were observed for C*RC, WC*RC, P*SC and PR*RC but no significant interaction for three years' average CP content. The modelling of individual harvest data for CP content over 23 harvests revealed 81 significant interactions out of 345 occurrences across the binary mixtures. The significant quadratic model for an average of three years of CP content data was used to create a contour plot and response surface diagram, showing the component species proportions for higher CP herbage biomass. The darkest area in Figure 4.3 (contour plot) produced lower CP content, through increasing the PR proportion in the mixture.

Equation 4.2 Quadratic model for Crude Protein (CP)

CP = 16.85 PR + 18.62 C + 21.64 P + 23.73 WC + 24.31 RC + 20.72 SC – 5.7 PRC – 5.91 PRP – 5.96 PRWC + 0.56 PRRC – 8.17 PRSC – 4.14 CPI – 3.89 CWC + 2.26 CRC – 2.35 CSC – 5.36 PWC – 0.99 PRC + 1.61 PSC + 1.58 WCRC + **11.01 WCSC** + **9.2 RCSC** (R^2= 95.4%, $P < 0.01$).



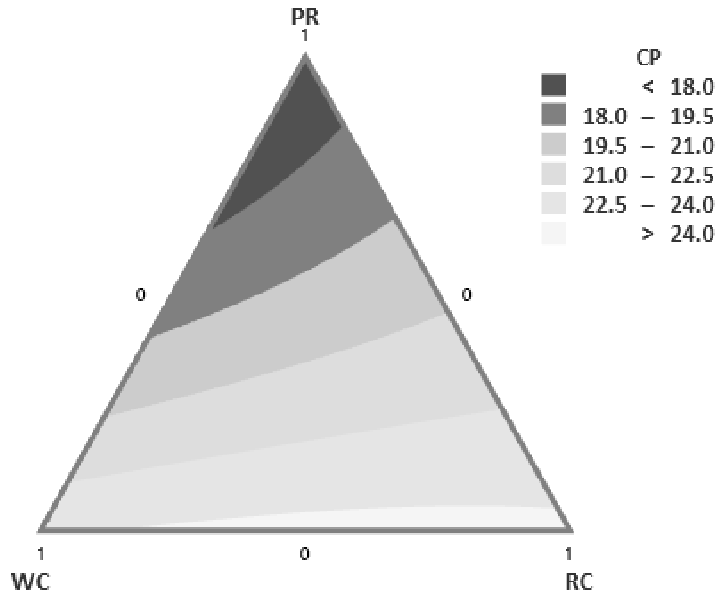


Figure 4.3 Ternary contour plot and response surface diagram for three years of average CP content from a quadratic model fitted to the function of the sown proportions of PR, WC and RC from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Diversity effect on Metabolizable Energy (MJ ME/kg DM)

The significant linear component of the quadratic model ($p < 0.01$) indicated differences in the ME content of monoculture swards. The proportion of species in the mixture impacted ME content of mixtures ($P < 0.001$). The ME content of the monocultures ranged from 10.6 MJ ME/kg DM for red clover to 11.3 MJ ME/kg DM for perennial ryegrass. The average metabolizable energy content of the monoculture species, binary and centroid mixtures is given in Table 4.11. The linear component of the quadratic model revealed that PR had the highest ME content, which significantly differed among species. The model had an adequate fit (R^2 = 82.3%; adj R^2 (adj)= 75.7%). The mixture combinations such as PR*WC, PR*RC, and WC*SC showed significant interaction as highlighted in Equation 4.3. The negative and less than one (<1) coefficient values indicate that the mixing of species did not improve the ME content of herbage material (Appendix D).

Equation 4.3 Quadratic model for ME content

ME = 11.24 PR + 10.66 C + 10.86 P + 10.71 WC + 10.53 RC + 10.77 SC + 0.16 PRC – 0.21 PRP +**0.46 PRWC** – **0.31 PRRC** + 0.65 PRSC + 0.10 CP + 0.34 CWC + 0.12 CRC + 0.27 CSC + 0.32 PWC -0.02 PRC – 0.09 PSC + 0.31 WCRC + 0.31 WCRC + **0.98 WCSC** + 0.04 RCSC. (R^2= 82 %, R^2(adj)= 75.7%, $P < 0.01$).

The significant interaction PR*WC component was mixed with RC to create a triple mix component. The downward contour lines moving towards PR showed that increasing PR proportion in the mixture improves the ME content. The darkest region towards PR and along the edge of PR*WC gave higher ME content herbage biomass. The pairwise interaction coefficient values were less than one and no significant interaction were found between the component species of binary mixtures except for PR*WC, PR*SC, and WC*SC. The darkest area of the contour plot represents the respective proportion of component species that improved the ME content of herbage biomass no interaction between component species. The modelling of sown proportion to the average ME content in each growth cycle rarely expressed the significant interaction between PR*WC (5 times out of 23 growth cycles) and PR*RC (one time out of 23 growth cycles).

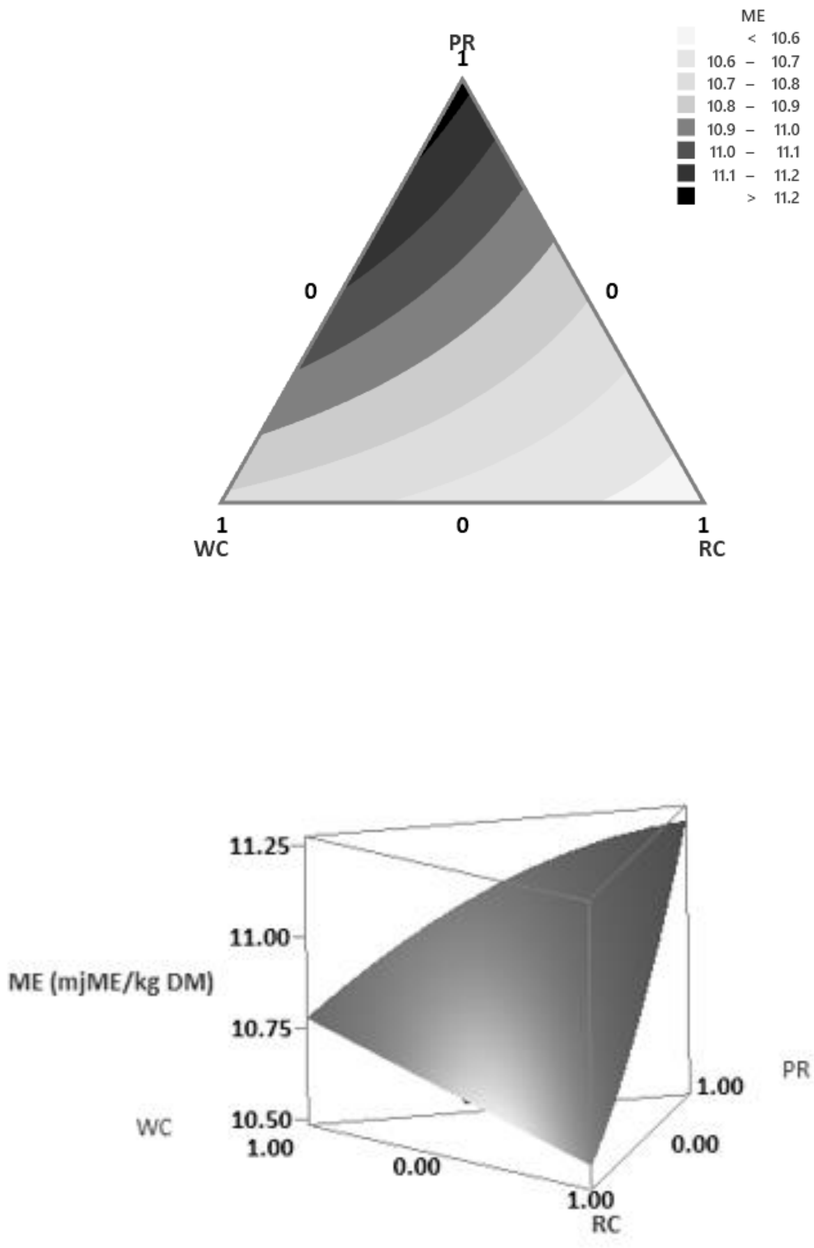


Figure 4.4 Ternary contour plot and response surface diagram for ME content from a quadratic model fitted to the function of the sown proportions of PR, WC and RC at Lincoln University, Canterbury, New Zealand.

4.4.4 **Pasture yield and quality persistence over the experimental period**

Table 4.9 The modelled DM yield of monocultures obtained from the linear model for 2018/19 to 2020/21 at Lincoln University, Canterbury.

Season	Perennial ryegrass	Cocks foot	Plantain	White clover	Red clover	Sub clover	P value	R^2	R^2 (adj)
2018/19									
Spring	1.54	1.05	1.07	1.27	1.32	1.65	0.000	26 %	23 %
summer	2.39	2.14	2.70	2.56	3.83	1.74	0.000	37 %	35 %
Autumn	2.56	1.92	1.97	1.61	1.82	1.53	0.000	25 %	23 %
2019/20									
Spring	1.69	1.51	1.30	1.50	1.56	1.65	0.000	34 %	33 %
summer	1.62	1.73	1.76	1.28	2.62	1.49	0.000	31 %	29 %
Autumn	2.07	1.77	1.60	1.52	1.60	0.99	0.000	22 %	20 %
2020/21									
Spring	2.02	1.40	1.74	1.99	2.33	1.65	0.000	47 %	39 %
summer	2.36	1.87	2.81	2.95	4.03	2.24	0.001	40 %	39 %
Autumn	1.95	1.23	1.12	1.13	1.31	1.08	0.040	16 %	14 %

Higher coefficient values are highlighted.

The primary productivity of the pasture response is the herbage biomass production, which is the most relevant relevance to the agronomic system (Kirwan et al., 2007). The seasonal average biomass yield was modelled to the initial sown proportion of the mixtures. The significant linear model ($P<0.04$) showed that the estimated biomass yield of pasture species in monocultures differed among species, and significant seasonal variation also existed ($P <0.01$). The data showed a considerable variation in the fitted models (16 to 47%). The SC produced the lowest estimated biomass yield of 0.99 t/ha in autumn 2019/20, and RC produced the highest value of 4.03 t/ha in summer 2021. Over three years, the average estimated seasonal biomass yield for SC, C, P, WC, and PR was 1.55, 1.62, 1.78, 1.75, and 2.02 t/ha, respectively. The estimated monoculture yield of cocksfoot remained consistent for the first and second years (1.61 and 1.62 t/ha) and reduced to 1.28 t/ha in 3rd year.

Table 4.10 The interaction coefficient values obtained from modelling sown species proportion on average seasonal biomass yield from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

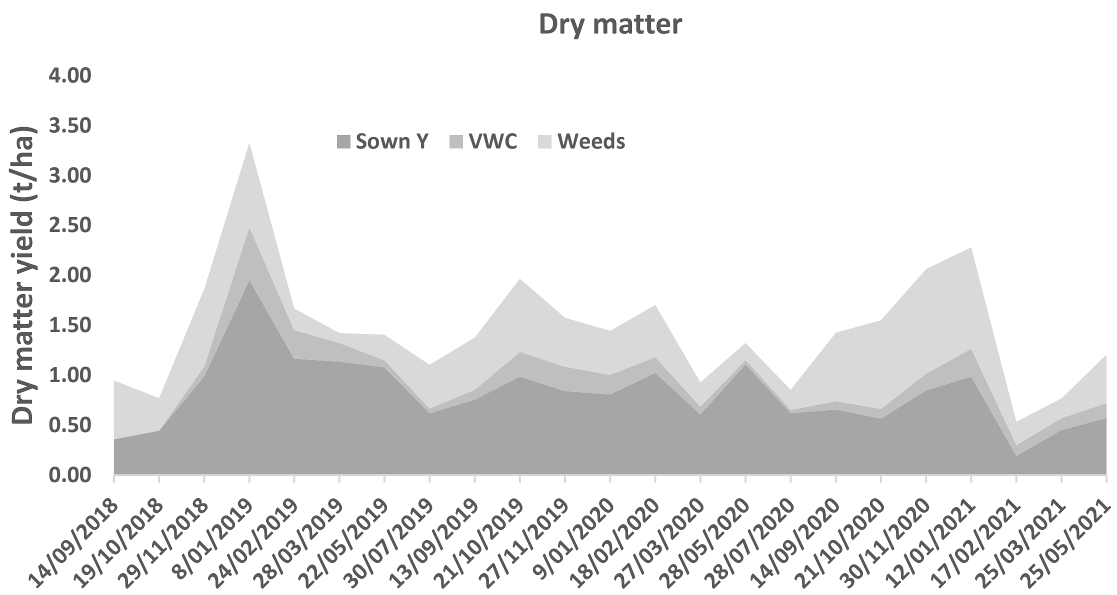
Binary mixture	18/19 spring	18/19 summer	18/19 autumn	19/20 spring	19/20 summer	19/20 autumn	20/21 spring	20/21 summer	20/21 autumn
PR*C	-0.05	-0.69	0.82	0.51	0.49	0.13	**2.05**	**2.84**	0.45
PR*P	0.43	-1.03	-0.01	**0.95**	1.04	0.74	**1.49**	**3.22**	0.69
PR*WC	-0.14	0.03	**1.60**	-0.07	-0.46	**2.52**	**1.41**	**3.35**	**3.86**
PR*RC	**1.18**	1.60	**2.53**	-0.39	**1.99**	1.37	**2.69**	**7.46**	**2.47**
PR*SC	**2.30**	0.22	**4.21**	-0.13	-1.39	**4.26**	0.92	0.09	**1.60**
C*P	0.04	1.23	0.56	0.28	1.20	0.81	**1.27**	**2.94**	0.62
C*WC	0.52	1.50	0.93	0.07	0.46	**2.20**	0.86	**2.78**	**1.85**
C*RC	**1.04**	**4.07**	**1.84**	**1.39**	**2.60**	**3.04**	**1.99**	**4.74**	**1.68**
C*SC	0.69	-0.48	**3.48**	0.10	0.60	**2.13**	-0.77	**-3.85**	0.12
P*WC	-0.16	0.30	**1.68**	0.60	**1.91**	0.32	0.12	0.32	0.36
P*RC	-0.68	0.27	0.80	-0.29	**1.88**	-1.20	0.74	1.40	-0.10
P*SC	-0.12	1.31	**2.03**	-0.49	-0.25	**1.93**	-0.09	-0.97	-0.36
WC*RC	0.64	**2.41**	1.23	0.17	**2.11**	1.15	0.20	1.64	0.21
WC*SC	0.63	-1.44	**2.55**	0.17	-0.10	**1.95**	0.68	2.31	**1.28**
RC*SC	-0.05	**2.24**	**1.64**	-0.36	-0.06	**1.58**	0.65	2.14	-0.02
P Value	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000
R^2	38%	47%	49%	39%	39%	42%	51%	56%	33%
R^2 (adj)	32%	42%	44%	34%	33%	37%	47%	52%	27%

The seasonal biomass yield as a function of the initial sown proportion showed variation in the diversity effect. The quadratic model analysis showed that the blending proportions in the mixture significantly influenced biomass yield. A consistent interaction pattern was observed in the mixture communities such as PR*WC, PR*RC and C*RC. The seasonal interaction coefficient values from the quadratic model differed with associated component species and the season in each re-growth cycle. The interaction coefficient values from the quadratic model are presented in Table 4.10.

DM production of Sown and unsown (VWC/weeds) fraction in monoculture and species mixed swards

Figure 4.5 shows the sown and unsown biomass yield fraction of monoculture (top) and species mixed pasture swards (bottom). The volunteer white clover and weeds were considered as unsown species. This comparison gave a common focus of sown species' persistence over time. The area in the graph represents the sum yield of the above ground components over three years. The dark grey area indicated the sown yield, and cloudy grey and milky grey colour bands indicated the VWC and weed fractions, respectively. The fraction of biomass production of monocultures and species mixed swards showed seasonal variations. The seasonal peak biomass production in monoculture and mixture communities was in late spring to early summer. The band of grey and light grey area on the top of the dark grey area (sown species biomass) was higher for monocultures than the species mixed swards. This indicates that the monoculture swards allowed more room for the growth of unsown species. An overall comparison revealed that the mixture communities produced 35% lower weed biomass than the monoculture average. Regardless of functional species, monoculture swards produced a sum biomass yield of 33.5 tons, and the diverse species pasture swards produced 39.1 tons over three years. An annual variation of sown and unsown biomass yield proportion is shown in Table 4.13.

In monoculture swards, the total biomass yield of the sown species declined overtime. It dropped from 7.1 t/ha in the first year to 4.9 t/ha in the third year. Meanwhile, the biomass of VWC and weed biomass in the sward increased annually. The average sown biomass yield of mixture communities ranged from 10.8 tons in the first year to a maximum of 11.5 tons in the second year. In diverse species mixed pasture swards, the total yield of VWC was increased each year continuously from the establishment cycle year (0.39 t/ha) to the third year (0.59 t/ha), giving a total VWC biomass yield of 1.41 tons over three years.



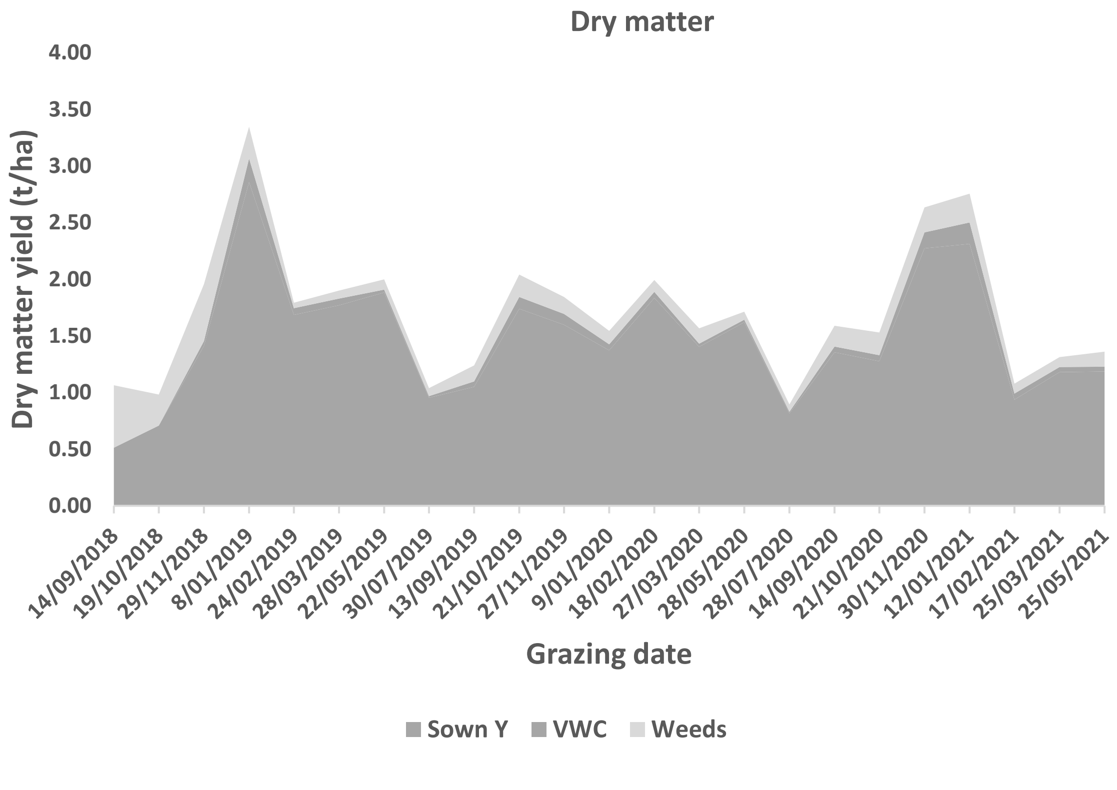


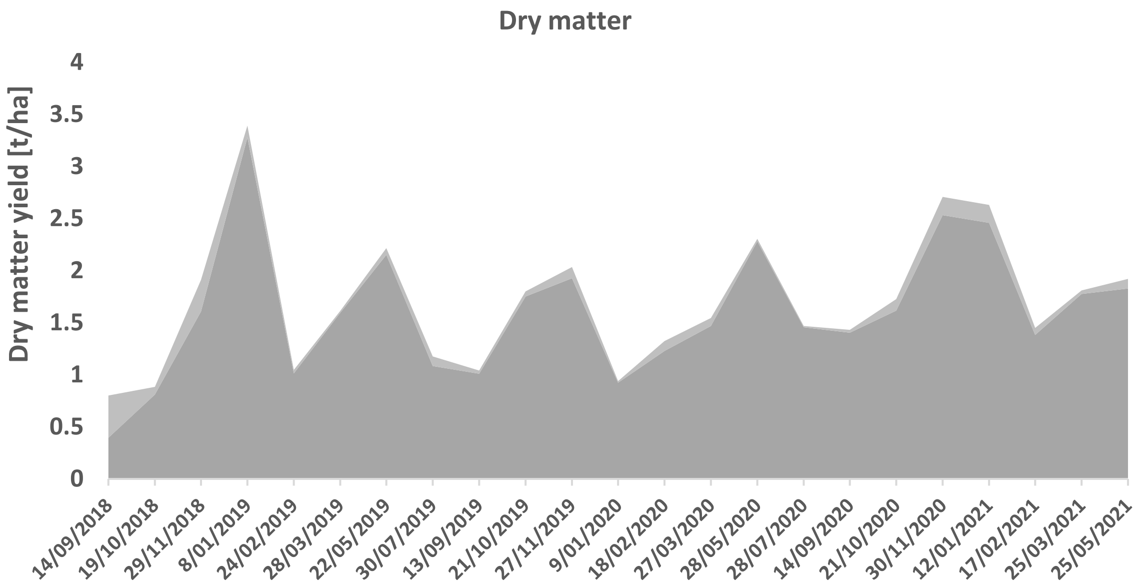
Figure 4.5 Dry matter production of sown and unsown (weed and VWC) species fraction across the monocultures and species mixed swards in a pasture diversity experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

DM production of sown and unsown species in PR*WC, PR*RC and PR*WC*RC mixtures

The contribution of sown and unsown species to the total biomass yield of mixture communities such as PR*WC, PR*RC and PR*WC*RC are shown in Figure 4.6 and Figure 4.7. The sum of annual biomass yield of the PR*WC continuously increased from establishment (11 t/ha) to the third year (14 t/ha). Weed biomass yield fluctuated from 1.1 t/ha in the first year to the lowest biomass yield of 0.49 t/ha in the second year and 0.69 t/ha in the third year. This sward maintained the sown species biomass yield over time.

Similarly, PR*RC also maintained the sown biomass yield over time. Breaking down the total sown biomass yield into annual sown biomass yield revealed that sown yield was not different in the first and second years (12t/ha) and 13.9 t/ha in the third year. VWC biomass yield increased annually. In the first, second and third years, they were 0.46, 0.52 and 1.28 t/ha. Weed biomass yield decreased from 1.14 t/ha in the establishment year to 0.56 and 0.60 in the second and third year, respectively. The highest weed biomass yield of 1.07 t/ha in spring in the establishment year. This mixture gave a lower weed biomass yield of 0.24 and 0.26 t/ha in the spring and summer in 2020/21, while the VWC biomass yield was 0.67 and 0.31 t/ha in the above seasons, respectively. The highest sown biomass yield of PR*RC was achieved at the end of spring and the beginning of summer in each production year (Figure 4.6).

The PR*WC*RC ternary mixture produced a total biomass yield of 42.8 t/ha, with the sum of sown species yielding 40.6 t/ha. The above mixture produced a total annual sum sown biomass yield of 14.2 t/ha, 12.6 and 13.7 t/ha in the first, second and third years, respectively. Figure 4.7 shows this mixture maintained the biomass yield of sown proportions. The seasonal total sum yield across the experimental period in spring, summer and autumn were 15.4, 14.4 and 10.8 t/ha.



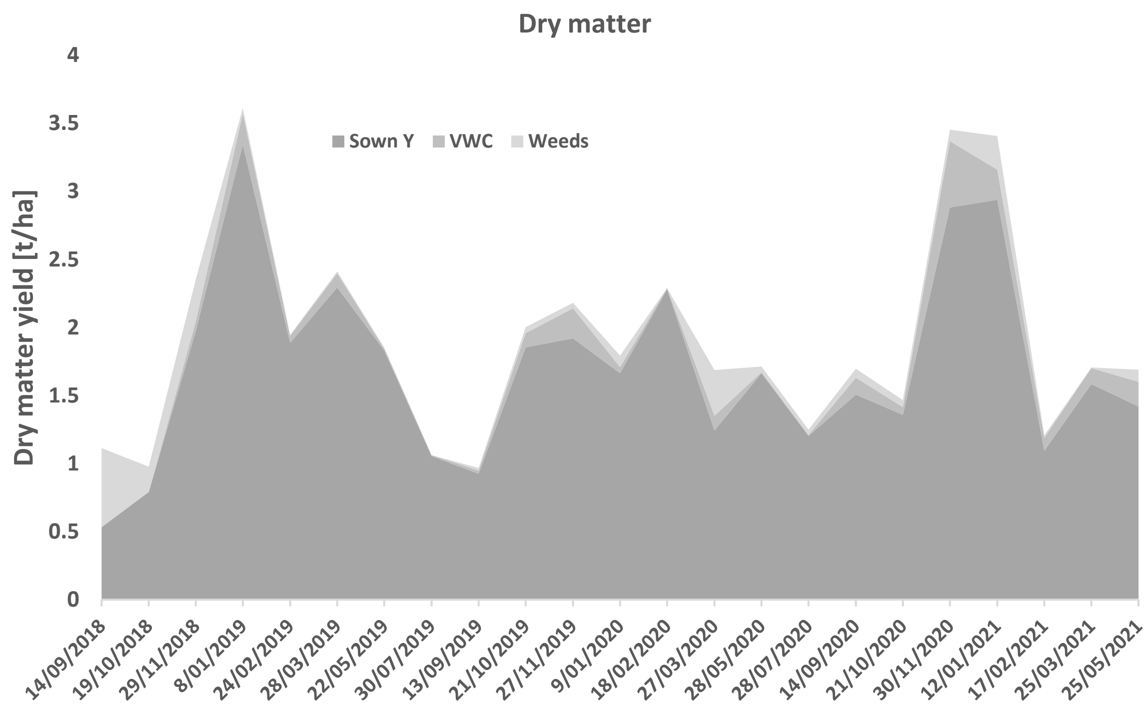


Figure 4.6 Dry matteryield of sown species and unsown species (weed and VWC) fraction in PR*WC (0.5:0.5) (top) and PR*RC (0.5:0.5) (bottom) mixtures in a diverse species mixed pasture experiment at Lincoln University, Canterbury, New Zealand (2018-2021).

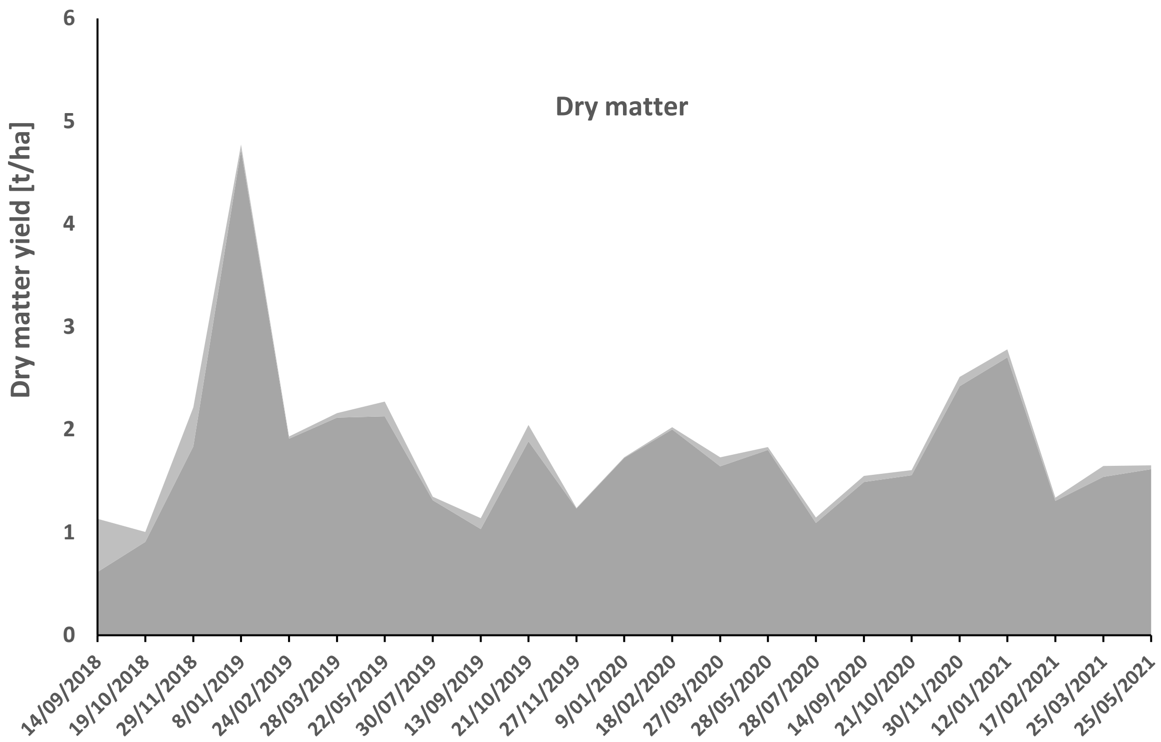


Figure 4.7 : Biomass yield of sown species and unsown species (weed and VWC) fraction in PR*WC*RC ternary mixture at the equal proportion component species in a diverse species mixed pasture experiment at Lincoln University, Canterbury, New Zealand (2018-2023).

Crude protein (CP) and Metabolizable (ME) Energy content of monocultures and binary mixtures over time

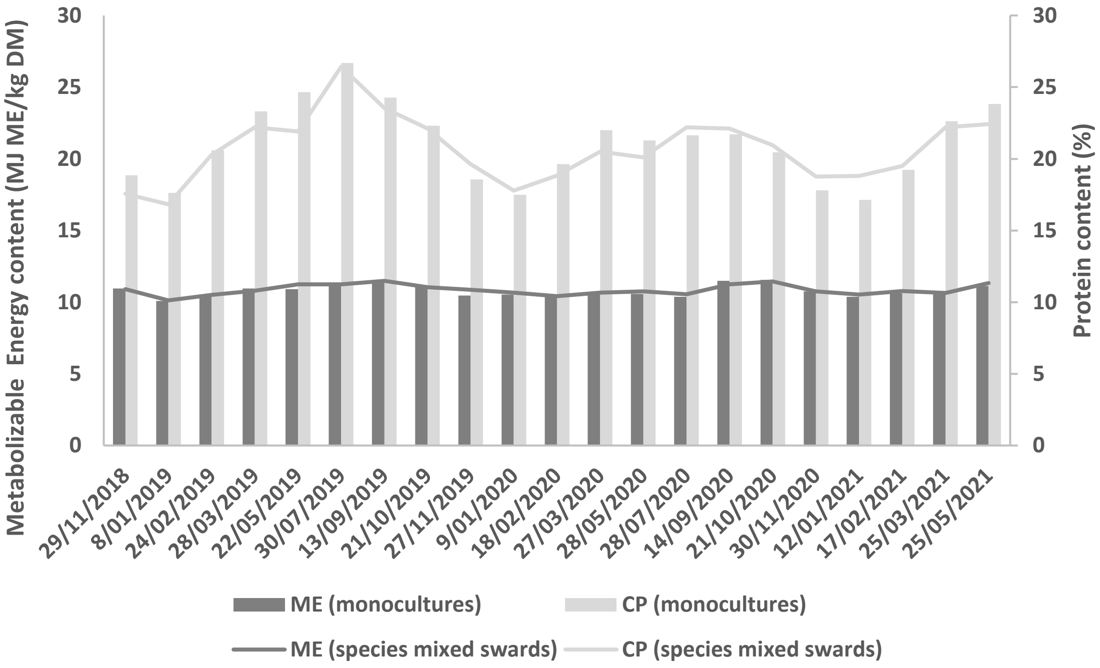


Figure 4.8 Crude Protein (CP) and Metabolizable Energy (ME) content of the monoculture and species mixed swards over grazing cycles from the 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

CP and ME content of monoculture and species mixed swards herbage over time is shown in Figure 4.8. The ME and CP content of monocultures ranged from 10.1 - 11.5 MJ ME/kg DM and 17.2 -26.7%, respectively. The species mixed swards did not show greater variation; ME and CP content ranged from 10.1 to 11.5 MJ ME/kg DM and 16.8 to 26.4%, respectively. Mixing pasture species to formulate diverse pasture swards for pasture quality improvement, especially for CP and ME, was discussed in Section 4.4.3. On average, ME content of the monocultures and diverse species mixtures was 10.8 MJ ME/kg DM and did not show a greater variation between autumn and spring. The monocultures' average ME content in spring, summer and autumn were 11.0, 10.4 and 10.8 MJ ME/kg DM. The average CP content of species mixed swards in summer, autumn, and spring were 18.7, 21.5 and 21.5%. The mixing of legume components improved the protein content of the swards. The highest CP content was from mixing two legume component species (Table 4.11).

4.4.5 Numerical optimization

Pasture sward responses such as DMY, DMY-W, ME and protein were simultaneously considered in the optimisation of component species. In the quadratic model, non-significant terms were removed and reanalysed for model improvement. An improved quadratic model was subjected to a response optimizer in MINITAB 20. The response optimizer considered all parameters simultaneously and gave the optimal solution for the component species proportion in the seed mixture. PR*RC was one of the identified mixtures strongly influencing economically important pasture sward responses over the regrowth cycles. Moreover, the identified ternary mixture PR*WC*RC, showed an interaction at P=0.08 with higher coefficient values for the three year average response, indicating that the mixture's estimated responses were higher than the monoculture responses for DMY (Biomass yield /ha/growth cycle), and DMY-W (weed biomass yield).

These numerical multiple response optimization for PR*RC results predict the maximum DMY and lower weed biomass yield with higher CP and ME content.

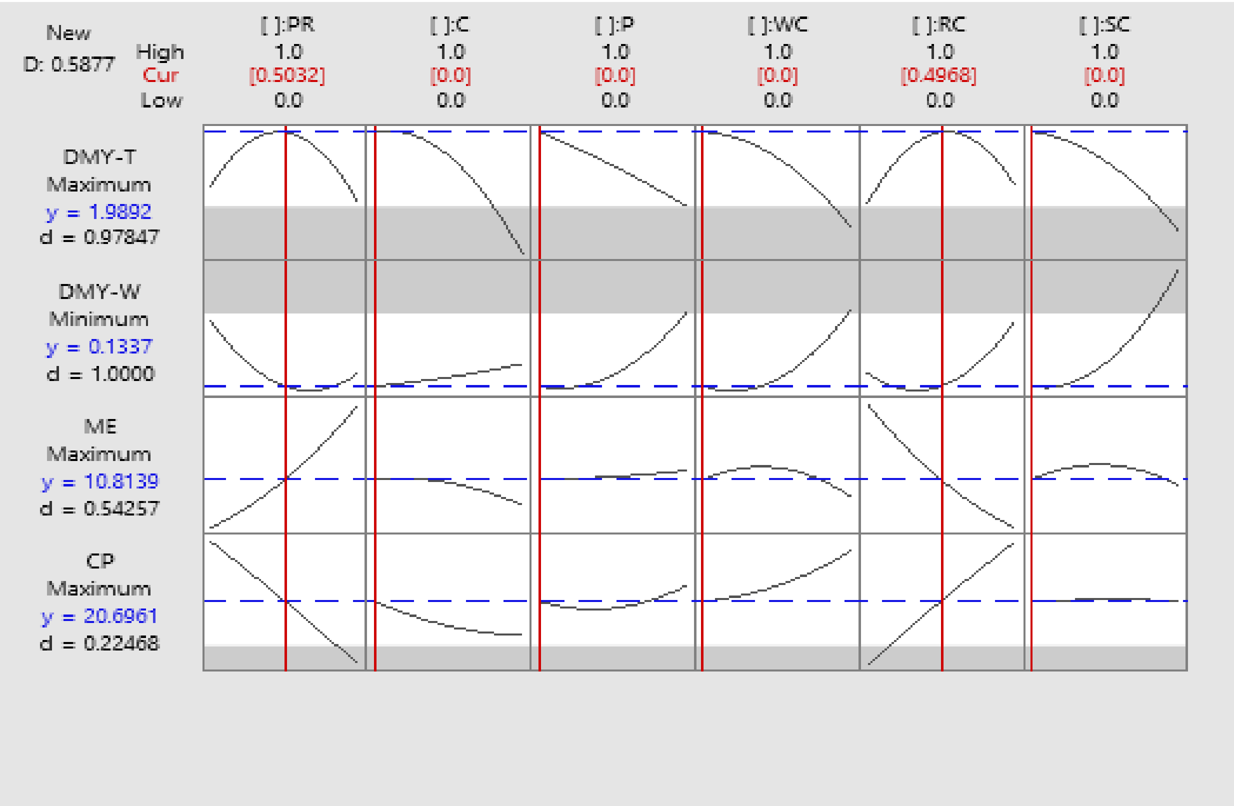


Figure 4.9 Response optimizer for PR: RC component settings for optimization of DMY-T (above ground biomass), DMY-W (weed biomass), ME (metabolizable energy), and CP (crude protein) content developed in Minitab 20 statistical software at Lincoln University, Canterbury, New Zealand.

PR:RC = 50:50 was one of the component species settings that optimized the dry biomass yield 1.98 t/ha, less weed biomass 0.13 t/ha, 10.8 MJ ME/kg DM and CP 20.7%. The species proportion in the binary mixture was equivalent to 12.6 kg PR and 13.4 kg RC. This was the composite most desirable in optimizing responses for binary component species (PR*RC) proportion settings. The highest weed suppressive effect and highest ME content could be achieved by increasing the PR and reducing the RC proportion in the mixture. In contrast to the above component species proportion adjustment, reducing the PR and increasing the RC proportion in the mixture improves the CP content. These components exerted the opposite effect on ME content. For example, the higher RC content increased the protein content, whereas a higher proportion of PR reduced CP content of herbage. In addition to PR*RC optimisation, PR*WC*RC ternary mixture at two proportional settings (Figure 4.10 and Figure 4.11) improved pasture production.

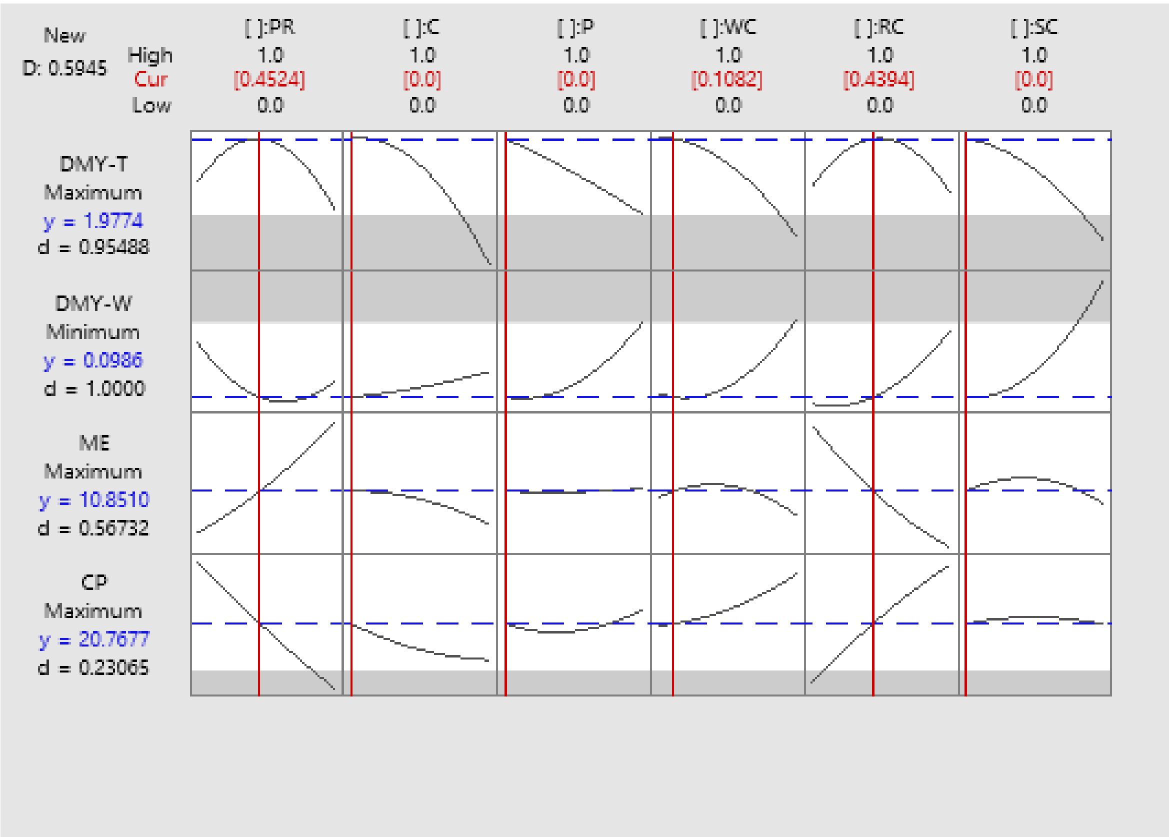


Figure 4.10 Response optimizer for PR:WC:RC component settings for higher DMY-T, lower DMY-W, improved ME and CP content from a diverse species mixed pasture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Figure 4.10 shows the optimization PR*WC*RC at the ratio of 45:11:44 produced DM yield of 1.97 t/ha, weed biomass 0.09 t/ha, 10.85 MJ ME/kg and CP 20.8%. This species proportion was equal to a seed rate of 11.3 kg/ha PR, 0.8 kg/ha WC and 11.8 kg/ha RC. PR and RC improved the DM yield by a greater proportion, as shown in the variate settings (Figure 4.10). Similarly, further addition of WC reduced the biomass yield and increased CP content. Based on the modelled and interactive plot, it was predicted that an increasing WC proportion would allow more weed invasion (higher weed biomass) invariably correlated with reduced biomass yield and ME content. The increased PR proportion would control weed invasion and improve the ME content (Figure 4.11). In contrast to the improvement of ME content, increasing PR proportion had a negative effect on CP content and DM yield. In addition to the above ternary mixture proportion setting, Minitab 20 statistical software calculated the optimal solution for higher DM yield, lower weed biomass, improved CP, ME, ADF and NDF as given in Figure 4.11.

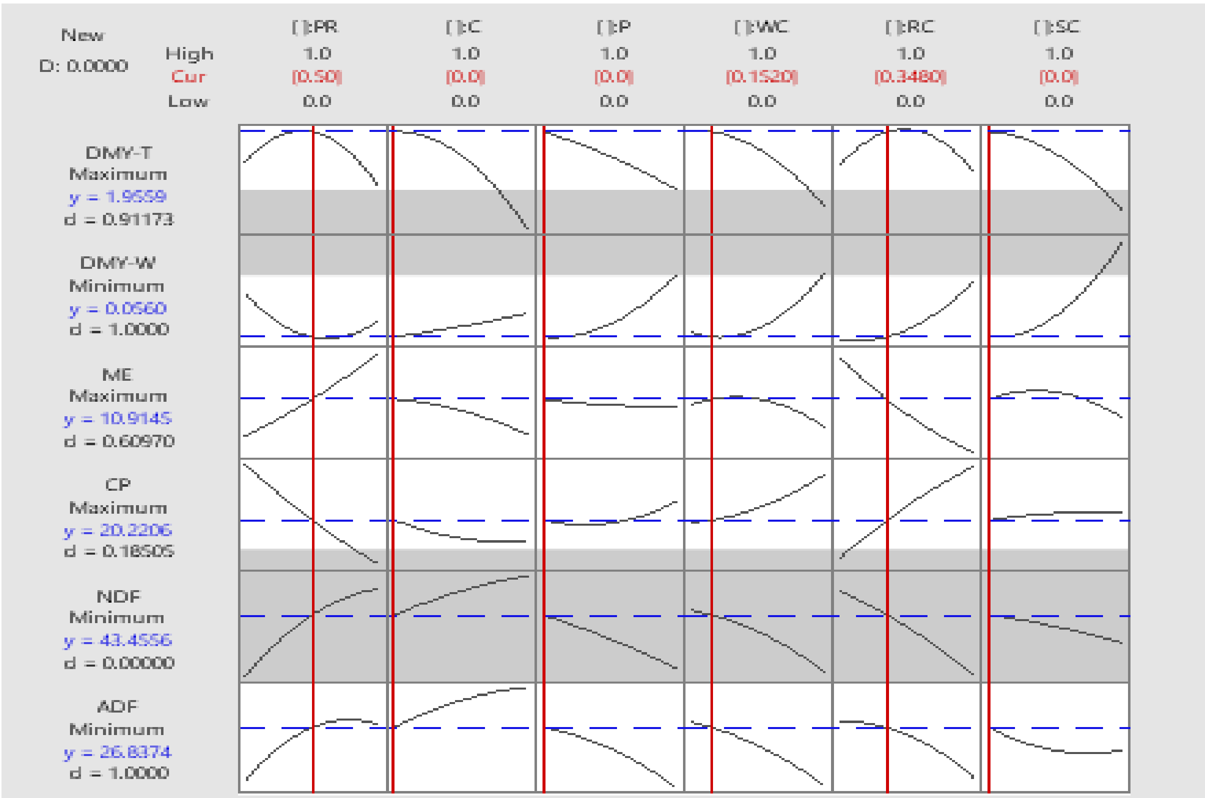


Figure 4.11 Response optimizer for PR:WC:RC component settings for improved pasture responses such as DMY-T, DMY-W, ME, CP, NDF and ADF content in a multi-species pasture mixture experiment at Lincoln University, Canterbury, New Zealand.

PR*WC*RC in 50:15:35 could maximize the responses with the desired ADF and NDF concentration of 26.8 and 43.4%, respectively. Increasing PR proportion in the mixture increases ADF and NDF per cent and reduces the sward's CP content. PR*WC*RC combination at the ratio of 50:15:35 equivalent to the seed rate of 12.6 kg/ha PR, 1 kg WC and 9.4 kg/ha RC produced a higher DM yield of 1.96 t/ha, lower weed biomass 0.06 t/ha, 10.9 MJ ME/kg DM, CP 20.2%, NDF 43.45% and ADF 26.8%.

4.4.6 Contribution of functional components to the diversity

Functional group model analysis (DI model) captures the contribution of the functional trait diversity on sward responses, considering DM as an important agronomic parameter. The experimental set-up targeted to test the species proportion was re-arranged based on functional groups and analysed to determine the effect of the functional group species on herbage biomass yield. The modelled herbage biomass yield of the functional component group differed ($P < 0.01$), and their proportions in binary composition improved the herbage biomass yield and reduced the weed biomass yield. The model yield of functional species such as annual legume (AL), Plantain (P), Grasses (G) and perennial legumes (PeL) were 1.48, 1.59, 1.71 and 1.79 t/ha, respectively. The quadratic model ($P = 0.01$) revealed that the contribution of functional species proportion on herbage biomass yield and weed suppression was significant (Equation 4.4 and Equation 4.5). The higher potential DM yield was from combining the grass functional group (PR or C), and perennial legumes which gave a yield advantage of 3.6 t/ha over the experimental duration. The model for three years' average biomass yield had a reasonable fit (30.6%) to the data, but for weed biomass, 71% of the variation in weed biomass yield depended on functional group proportions.

Equation 4.4 Quadratic model analysis for herbage biomass production of functional species

DMY = 1.43 G + 1.32 AL + 1.58 PeL + 1.48 P + **0.84 G*AL** + **1.34 G*PeL** + **0.75 G*P** + 0.054 **AL*PeL** + 0.28 AL*P + 0.31 PeL*P (R-sq = 34%, R-sq(adj) = 31%, p =0.00.)

Equation 4.5 Cubic model analysis for weed biomass yield of functional species.

DMY-W = 0.27 G + 1.12 AL + 0.62 PeL + 0.74 P **– 1.78 G*AL - 1.12 G*PeL - 0.71 G*P – 0.94 AL*PeL – 1.22 AL*P – 1.25 PeL*P – 1.76 G*AL*PeL** – 0.05 G*AL*P **– 2.02 G*PeL*P – 2.75 AL*PeL*P** (R-sq = 71.3%, R-sq(adj) = 71%, p =0.01.), (highlighted functional group mixtures shows significant interaction at p=0.05).

The significant linear model revealed that the monoculture functional species group suppressed the weed growth and produced a weed biomass yield of - 0.20 t/ha in the grass functional component to 0.61 t/ha in annual legumes. The quadratic and cubic models were significant ($P<0.01$), indicating that the mixture's functional group proportion reduced the weed biomass yield in binary and ternary mixtures.

4.5 Discussion

Pasture herbage biomass production with high quality is often the most important expected outcome of pastoral farming, which can be obtained from blending pasture species. Diverse pasture seed mixture formulation requires interdisciplinary knowledge about monoculture performance and the species' performance in a mixture (mixture behaviour). This chapter has two significant contributions to achieve the overall aim of formulation of productive pasture seed mixtures: (1) exploring the diversity contribution of mixture combinations formulated from six common pasture species and (2) optimising the most compatible species for improved yield and quality over time. In formulation of diverse species mixed pasture swards, some key questions have been overlooked to maximise production targets. In particular research is needed into which specific species and how many species need to be included in the mixture and, their relative proportions to improve dry matter yield and quality over time. This multi-species mixture experiment identified the optimal proportion of pasture species mixture as an alternative, promising tool for improving pasture production under irrigated and zero nitrogen fertilizer application conditions. The main statistical procedure considered is that the pasture community response depends on the proportion of mixture components in response surface modelling. The optimisation of pasture species proportion is the last step of the response surface model analysis.

Diverse species mixed pasture swards in NZ have potential for yield improvement and weed suppression with an improvement of herbage quality compared with monocultures (Black et al., 2017; Ryan-Salter and Black, 2012). The pasture mixture experiment identified several mixture combinations that improved biomass production and weed suppression. Herbage biomass production increased with the increasing number of species in the mixture and reached a peak with up to the inclusion of five species in the mix and levelled off with a further increasing number of species in the mixture. On average, mixture communities produced a higher yield (1.70 t/ha; 13.6 t/ha/yr) than the average of monocultures (1.46 t/ha; 11.7 t/ha/yr). Around half of the mixture communities (48%) reduced the weed biomass less than the most suppressive monoculture species which was perennial ryegrass 0.02 t/ha (1.6 t/ha). The pasture diversity experiments revealed that the species richness in the sward increased the above-ground biomass yield due to the contribution of productive species in the mixture (identity) and diversity contribution (Sanderson, 2010).

Diversity attributed to pasture productivity begins with combining the different functional species groups in the mixture, quantified through quadratic modelling. Functional model analysis for herbage biomass and weed yield was significant ($P<0.01$) and provided the general idea of blending functional groups for beneficial biomass yield and weed suppression. The coexistence of the functional groups may be due to niche partition and facilitation (Andrews et al., 2011; Black et al., 2017; Connolly et al., 2011). The mixture effects differed in magnitude and direction based on the associated species combination, season and pasture response (Kirwan et al., 2009). The quadratic model for functional groups identified that combining the functional components, grasses, and perennial legumes exhibited the highest diversity contribution for herbage biomass production and weed suppression. The diversity contributed to beneficial effects due to the inclusion of key functional species in the mixture, mainly the grasses and legumes, improved biomass production (Grime, 1997; Huston, 1997) and weed suppression (Barker et al., 1999). The robustness of the diversity contribution over time was observed from PR*WC, PR*RC, and the ternary mixture PR*WC*RC. VeliegherDe and Carlier (2005) also reported that greater contribution from PR*WC and PR*RC binary mixtures. The yield improvement may be due to niche complementation for growth resources and facilitation in pasture mixture community compared with monocultures (Hector et al., 1999; Tilman, 1999).This is investigated further in Chapter 6.

The interspecific interaction in species mixed pastures quantified in this experiment is dynamic by nature and can be manipulated by changing pasture composition. Modelling of three years of average DM yield against the sown proportion quantified the highest diversity effect in PR*RC, PR*WC, C*RC, PR*C*P, PR*WC*RC and P*WC*RC. These combinations had strong interactions between species and provided higher ecosystem services in a simple mixture community at two to three species mixture levels. The quadratic and cubic model analysis confirmed that the mixture effect and identity contribution were the main community properties that improved biomass yield and weed suppression (Sanderson, 2010). Therefore, this experiment identified the optimal seed mixture as 2 to 3 species from PR, WC and RC. Other pasture mixture experiments that have identified 2-3 species from different functional groups (legume and non-legume) increased the herbage biomass yield and suppressed weed growth (Black and Lucas, 2018; Black et al., 2021; Myint et al., 2019; Ryan-Salter and Black, 2012). The interaction coefficient values for biomass production were positive in this mixture experiment. A wide range of compatible species in binary and ternary mixtures have been identified with various degrees of interaction contributions.

The interaction coefficient value can be positive, negative, or neutral (Kirwan et al., 2009). Among the mixture communities, over-yielding (higher than average monoculture yield) and transgressive overyielding (higher than the best-performing monoculture species) mixtures were identified. The interaction between species in binary mixtures contributed to DM production, which ranged from 1.38 to 11.7 t/ha over three years. The modelling of herbage biomass yield in each growth cycle showed that the interaction between the associated component species in PR*WC, PR*RC, and C*RC binary mixtures was significant over several regrowth cycles. The binary mixtures, PR*WC, and PR*RC diversity contribution produced a herbage biomass yield of 6.6 and 9.4 t/ha with considerable significant interaction (7 and 9 times, respectively, in 23 regrowth cycles) between component species. Similarly, binary combination C*RC also contributed an additional DM yield of 11.7 t/ha over the three years, but was not considered for optimisation because of the dominating characteristics of C in a mixture, lower herbage quality, palatability (Moloney, 1993) and difficulty to manage in the field (Mills, 2007; Moloney, 1993).

Plantain with perennial legumes, WC and RC in binary mixture combination gave the lowest diversity attributed to additional biomass production of 0.91 t/ha and 3 t/ha, respectively. The diversity effect on binary mixtures in PR*P and C*WC produced an excess biomass yield of 4.8 and 5.2 t/ha, respectively. The interaction between species in the binary mixture was not significant and showed a significant interaction only in four re-growth cycles. The interaction coefficient value for C*WC was 0.93. This mixture provided 5.23 t/ha during the experimental period and was much lower than the PR*RC. In the C*WC binary mixture, C exhibits above and below-ground spreading growth habits, and WC mainly competes for above-ground growth resources (Frame et al., 1998; Lorentzen et al., 2008). This competitive interaction between the species for growth resources reduced the ecosystem service and gave an interaction coefficient value less than one which shows reduced beneficial herbage biomass yield. Significant yield improvement and weed suppression were from PR*WC, and PR*RC binary mixtures. The refined cubic model with a positive higher interaction coefficient confirmed ($p=0.08$) that the species in the ternary mixture produced a DM yield of 12.9 t/ha. The higher productivity of the above mixtures may be due to the efficient utilization of growth resources (Sanderson et al., 2004) and synergistic interaction (Nyfeler et al., 2011).

The effect of species proportion on weed suppression was strongly significant (significant quadratic and cubic models at $P= 0.01$). The mixture contribution on weed suppression was quantified at 2 to 3-species mixture level, even though increasing species richness continuously suppressed the weed growth. The strong diversity effect on suppression of unsown species persisted over several regrowth cycles in PR*WC and PR*RC binary combinations. The identified binary and ternary mixtures (PR*WC, PR*RC, PR*WC*RC) maintained a higher proportion of sown species over three years.

Modelling of three years' average nutritive value revealed that the species proportion in the mixture influenced ($P < 0.01$) ME and CP content. The pasture plant diversity on different establishment methods in a four year continuous experiment showed that a similar response of species proportion in the mixture influenced ($P < 0.01$) nutritional composition (Black et al., 2021). However, the negative coefficient value of the interaction showed that mixture contribution did not improve nutritional composition. Pasture diversity experiments usually neglect the nutritional status of herbage biomass Hopkins and Holz (2006). For herbage quality improvement was mainly due to identity contribution rather than the diversity effect. Blending the legume and non-legume components improved the CP content of herbage material. The identity contribution to the community response is considered a passive contributor/descriptor of the community (Stirling and Wilsey, 2001). The number of species in the mixture did not influence pasture quality (Deak et al., 2007). The seasonal variation in nutritional quality was not significant in this experiment under irrigation. The average CP and ME content was lower in summer than in autumn and spring, and seasonal weather affected the nutritional composition (Baars et al., 1990). The nutritive value of herbage mainly depends on the botanical composition (Sturludóttir et al., 2014).

The numerical optimization identified that PR-based binary and ternary pasture mixture with perennial clovers WC, and RC improved the pasture yield and quality. The fitted model control the proportions of component species to maximise the responses. Binary mixtures PR:RC = 0.5:0.5 in proportions equivalent to 12.6 and 13.4 kg/ha seed rate produced 1.98 t/ha DM yield (16 t/ha), 0.13 kg/ha weed biomass, 10.8 MJ ME/kg DM with 20.7% protein content. The ternary mixture, PR:WC:RC at the ratio of 45:11:44 component species proportions equivalent to 11.3 : 0.8 : 11.8 kg/ha seed rate produced 1.97 t/ha DM yield (16 t/ha), 0.09 t/ha weed biomass, 10.8 MJ ME/kg DM and 20% CP concentration.

Alternate to the above component proportions at the ratio of 50:15:35 equal to the seed rate of 12.6 : 1 : 9.4 kg/ha produced the DM yield of 1.96 t/ha (16 t/ha), 0.06 t/ha weed biomass, 10.9 MJ ME/kg DM, 20.2% CP, 43.4 NDF and 26.8 ADF. The mixture's legume and non-legume ryegrass improved the CP and ME content of the herbage biomass (Baker and Younger, 1987). In the optimal mixture RG*WC*RC, the erect growth habit of PR and RC may efficiently utilize light (Easton et al., 1997) as an above-ground niche partitioning for light interception and N from legume component species may facilitate the higher herbage biomass yield. This will be investigated in Chapter 6.

4.6 Conclusion

- Species mixed pasture swards performed better than the monocultures, and several superior performance mixtures combinations have been identified for higher herbage biomass production with lower weed biomass yield. On average, across three years, species mixed swards gave 16% higher herbage biomass yield and 48% lower weed biomass than the most suppressive monoculture. The average nutritional composition of swards did not show variation. The legume proportion in the mixture improved the CP content of herbage materials.

- The DI interaction model tool identified that the species proportion in the mixture significantly affected the herbage biomass yield and weed yield. The significant effect was at 2 to 3 species level mixtures. Several binary and ternary mixture communities have been identified for improved biomass production and weed suppression. The mixture effect on herbage biomass production differed with the strength of the interaction; overyielding and transgressing overyielding mixtures were identified. A significant three-way interaction was also identified for herbage biomass yield and weed yield in the ternary mixture community.

- Modelling of annual biomass yield showed a higher diversity effect in the first and the third years than in the second year. The significant mixture effect on higher biomass productivity was in PR*WC, PR*RC and C*RC binary mixtures. These binary mixture communities maintained a significant interaction over several regrowth cycles.

- Mixture communities maintained a higher sown species proportion than the monocultures. Sown species proportions in the mixtures increased with increasing the number of species in the mixture. The sown species composition of monocultures differed among functional species. Grass functional components maintained a higher sown proportion than perennial legumes and plantain. The seasonal growth habit of the winter annual legume (sub-clover) facilitated the growth of unsown species. The identified mixture communities, such as PR*WC, PR*RC and PR*WC*RC, maintained a higher proportion of the sown species than monoculture component species.

- The diversity contribution to nutritional composition (ME/CP) was not beneficial, even though the species proportion significantly influenced nutritional composition. The pasture species mixed sward composition of legume and non-legume (grasses) improved CP content depending on the relative abundance of legume components in the mixture. The coefficient value less than one, and the negative coefficient value reveals that mixing the species did not improve the quality.

- Optimisation identified PR*RC (0.5:0.5), and two proportional settings of the PR*WC*RC (45:11:44 and 45:15:35) ternary mixtures improved higher DM yield, lowered weed biomass yield and improved nutritional quality. Those mixtures produced the average herbage biomass yield of 16 t/ha.

Table 4.11 Average yield and quality attributes of pasture swards from 2018 to 2021 in a multi-species pasture experiment at Lincoln University, Canterbury, New Zealand.

Mixture	PR	C	P	WC	RC	SC	Yield (t DM/ha)	Weed (t DM/ha)	ME (MJ/kg DM)	CP (% DM)
1	1	0	0	0	0	0	1.53	0.20	11.24	16.86
2	0	1	0	0	0	0	1.21	0.30	10.66	18.64
3	0	0	1	0	0	0	1.52	0.76	10.86	21.66
4	0	0	0	1	0	0	1.41	0.73	10.71	23.74
5	0	0	0	0	1	0	1.67	0.65	10.53	24.32
6	0	0	0	0	0	1	1.38	1.17	10.77	20.74
7	½	½	0	0	0	0	1.54	0.21	10.99	16.29
8	½	0	½	0	0	0	1.72	0.25	11.00	17.75
9	½	0	0	½	0	0	1.70	0.09	11.09	18.78
10	½	0	0	0	½	0	1.90	0.19	10.81	20.70
11	½	0	0	0	0	½	1.71	0.17	11.17	16.73
12	0	½	½	0	0	0	1.54	0.34	10.79	19.08
13	0	½	0	0	½	0	1.50	0.27	10.77	20.19
14	0	½	0	0	½	0	1.94	0.34	10.63	22.01
15	0	½	0	0	0	½	1.31	0.29	10.79	19.07
16	0	0	½	½	0	0	1.51	0.41	10.87	21.33
17	0	0	½	0	½	0	1.55	0.47	10.69	22.71
18	0	0	½	0	0	½	1.41	0.65	10.80	21.57
19	0	0	0	½	½	0	1.62	0.67	10.70	24.40
20	0	0	0	½	0	½	1.41	0.67	10.99	24.96
21	0	0	0	0	½	½	1.56	0.76	10.66	24.80
22	⅓	⅓	⅓	0	0	0	1.63	0.28		
23	⅓	⅓	0	⅓	0	0	1.56	0.11		
24	⅓	⅓	0	0	⅓	0	1.87	0.18		
25	⅓	⅓	0	0	0	⅓	1.48	0.16		
26	⅓	0	⅓	⅓	0	0	1.62	0.06		
27	⅓	0	⅓	0	⅓	0	1.78	0.15		
28	⅓	0	⅓	0	0	⅓	1.50	0.27		
29	⅓	0	0	⅓	⅓	0	1.86	0.09		
30	⅓	0	0	⅓	0	⅓	1.81	0.13		
31	⅓	0	0	0	⅓	⅓	1.88	0.22		
32	0	⅓	⅓	⅓	0	0	1.47	0.18		
33	0	⅓	⅓	0	⅓	0	1.68	0.23		
34	0	⅓	⅓	0	0	⅓	1.44	0.28		
35	0	⅓	0	⅓	⅓	0	1.80	0.26		
36	0	⅓	0	⅓	0	⅓	1.51	0.26		
37	⅓	0	0	0	⅓	⅓	1.68	0.34		
38	0	0	⅓	⅓	⅓	0	1.68	0.28		
39	0	0	⅓	⅓	0	⅓	1.59	0.39		
40	0	0	⅓	0	⅓	⅓	1.50	0.38		
41	0	0	0	⅓	⅓	⅓	1.59	0.50		
42	¼	¼	¼	¼	0	0	1.69	0.09		
43	¼	¼	¼	0	¼	0	1.74	0.18		
44	¼	¼	¼	0	0	¼	1.82	0.31		
45	¼	¼	0	¼	¼	0	1.97	0.10		
46	¼	¼	0	¼	0	¼	1.73	0.08		
47	¼	¼	0	0	¼	¼	1.84	0.22		
48	¼	0	¼	¼	¼	0	1.85	0.06		
49	¼	0	¼	¼	0	¼	1.64	0.09		
50	¼	0	¼	0	¼	¼	1.93	0.18		
51	¼	0	0	¼	¼	¼	1.74	0.08		
52	0	¼	¼	¼	¼	0	1.72	0.18		
53	0	¼	¼	¼	0	¼	1.60	0.16		
54	0	¼	¼	0	¼	¼	1.67	0.27		
55	0	¼	0	¼	¼	¼	1.61	0.25		
56	0	0	¼	¼	¼	¼	1.61	0.37		
57	⅕	⅕	⅕	⅕	⅕	0	1.86	0.12		
58	⅕	⅕	⅕	⅕	0	⅕	1.74	0.08		
59	⅕	⅕	⅕	0	⅕	⅕	1.76	0.22		
60	⅕	⅕	0	⅕	⅕	⅕	1.86	0.09		
61	⅕	0	⅕	⅕	⅕	⅕	2.05	0.09		
62	0	⅕	⅕	⅕	⅕	⅕	1.79	0.19		
63	⅙	⅙	⅙	⅙	⅙	⅙	1.84	0.06	10.85	20.66

Mix	PR	C	P	WC	RC	SC	PR	C	P	WC
64	7/12	1/12	1/12	1/12	1/12	1/12	2.04	0.06		
65	1/12	7/12	1/12	1/12	1/12	1/12	1.69	0.09		
66	1/12	1/12	7/12	1/12	1/12	1/12	1.87	0.09		
67	1/12	1/12	1/12	7/12	1/12	1/12	1.74	0.10		
68	1/12	1/12	1/12	1/12	7/12	1/12	2.00	0.10		
69	1/12	1/12	1/12	1/12	1/12	7/12	1.78	0.11		

Weighted average ME and CP concentrations with yield as the weight.
Emboldened values are above the average.

Table 4.12 Average botanical composition of pasture swards from a multi-species pasture mixture experiment at Lincoln University, Canterbury, New Zealand (2018 to 2021).

Mix	PR	C	P	WC	RC	SC	PR	C	P	WC	RC	SC	W
1	1	0	0	0	0	0	0.88	0.00	0.00	0.00	0.00	0.00	0.12
2	0	1	0	0	0	0	0.00	0.77	0.00	0.00	0.00	0.00	0.23
3	0	0	1	0	0	0	0.00	0.00	0.50	0.00	0.00	0.00	0.50
4	0	0	0	1	0	0	0.00	0.00	0.00	0.47	0.00	0.00	0.53
5	0	0	0	0	1	0	0.00	0.00	0.00	0.00	0.55	0.00	0.45
6	0	0	0	0	0	1	0.00	0.00	0.00	0.00	0.00	0.19	0.69
7	½	½	0	0	0	0	0.68	0.18	0.00	0.00	0.00	0.00	0.14
8	½	0	½	0	0	0	0.58	0.00	0.28	0.00	0.00	0.00	0.14
9	½	0	0	½	0	0	0.73	0.00	0.00	0.20	0.00	0.00	0.07
10	½	0	0	0	½	0	0.56	0.00	0.00	0.00	0.33	0.00	0.11
11	½	0	0	0	0	½	0.85	0.00	0.00	0.00	0.00	0.05	0.10
12	0	½	½	0	0	0	0.00	0.42	0.35	0.00	0.00	0.00	0.23
13	0	½	0	0	½	0	0.00	0.59	0.00	0.22	0.00	0.00	0.19
14	0	½	0	0	½	0	0.00	0.43	0.00	0.00	0.38	0.00	0.19
15	0	½	0	0	0	½	0.00	0.72	0.00	0.00	0.00	0.07	0.21
16	0	0	½	½	0	0	0.00	0.00	0.44	0.27	0.00	0.00	0.30
17	0	0	½	0	½	0	0.00	0.00	0.31	0.00	0.39	0.00	0.31
18	0	0	½	0	0	½	0.00	0.00	0.47	0.00	0.00	0.06	0.47
19	0	0	0	½	½	0	0.00	0.00	0.00	0.22	0.34	0.00	0.43
20	0	0	0	½	0	½	0.00	0.00	0.00	0.46	0.00	0.09	0.45
21	0	0	0	0	½	½	0.00	0.00	0.00	0.00	0.43	0.09	0.48
22	⅓	⅓	⅓	0	0	0	0.46	0.13	0.25	0.00	0.00	0.00	0.16
23	⅓	⅓	0	⅓	0	0	0.55	0.13	0.00	0.24	0.00	0.00	0.09
24	⅓	⅓	0	0	⅓	0	0.49	0.10	0.00	0.00	0.31	0.00	0.09
25	⅓	⅓	0	0	0	⅓	0.63	0.22	0.00	0.00	0.00	0.04	0.11
26	⅓	0	⅓	⅓	0	0	0.55	0.00	0.27	0.13	0.00	0.00	0.05
27	⅓	0	⅓	0	⅓	0	0.43	0.00	0.18	0.00	0.30	0.00	0.09
28	⅓	0	⅓	0	0	⅓	0.53	0.00	0.25	0.00	0.00	0.04	0.19
29	⅓	0	0	⅓	⅓	0	0.55	0.00	0.00	0.07	0.32	0.00	0.06
30	⅓	0	0	⅓	0	⅓	0.69	0.00	0.00	0.19	0.00	0.04	0.08
31	⅓	0	0	0	⅓	⅓	0.58	0.00	0.00	0.00	0.27	0.04	0.12
32	0	⅓	⅓	⅓	0	0	0.00	0.35	0.33	0.19	0.00	0.00	0.14
33	0	⅓	⅓	0	⅓	0	0.00	0.26	0.26	0.00	0.31	0.00	0.17
34	0	⅓	⅓	0	0	⅓	0.00	0.47	0.28	0.00	0.00	0.05	0.20
35	0	⅓	0	⅓	⅓	0	0.00	0.44	0.00	0.09	0.30	0.00	0.17
36	0	⅓	0	⅓	0	⅓	0.00	0.56	0.00	0.21	0.00	0.05	0.17
37	⅓	0	0	0	⅓	⅓	0.00	0.38	0.00	0.00	0.35	0.06	0.21
38	0	0	⅓	⅓	⅓	0	0.00	0.00	0.33	0.09	0.37	0.00	0.20
39	0	0	⅓	⅓	0	⅓	0.00	0.00	0.42	0.27	0.00	0.05	0.26
40	0	0	⅓	0	⅓	⅓	0.00	0.00	0.32	0.00	0.36	0.04	0.28
41	0	0	0	⅓	⅓	⅓	0.00	0.00	0.00	0.24	0.36	0.06	0.34
42	¼	¼	¼	¼	0	0	0.41	0.14	0.23	0.15	0.00	0.00	0.07
43	¼	¼	¼	0	¼	0	0.36	0.07	0.19	0.00	0.27	0.00	0.11
44	¼	¼	¼	0	0	¼	0.49	0.13	0.18	0.00	0.00	0.04	0.17
45	¼	¼	0	¼	¼	0	0.44	0.14	0.00	0.08	0.28	0.00	0.06
46	¼	¼	0	¼	0	¼	0.57	0.17	0.00	0.17	0.00	0.04	0.05
47	¼	¼	0	0	¼	¼	0.48	0.09	0.00	0.00	0.28	0.03	0.12
48	¼	0	¼	¼	¼	0	0.42	0.00	0.17	0.06	0.30	0.00	0.05
49	¼	0	¼	¼	0	¼	0.53	0.00	0.20	0.19	0.00	0.03	0.06
50	¼	0	¼	0	¼	¼	0.47	0.00	0.14	0.00	0.26	0.03	0.10
51	¼	0	0	¼	¼	¼	0.52	0.00	0.00	0.08	0.30	0.04	0.06
52	0	¼	¼	¼	¼	0	0.00	0.27	0.23	0.08	0.27	0.00	0.13
53	0	¼	¼	¼	0	¼	0.00	0.42	0.27	0.15	0.00	0.04	0.11
54	0	¼	¼	0	¼	¼	0.00	0.33	0.20	0.00	0.26	0.03	0.17
55	0	¼	0	¼	¼	¼	0.00	0.43	0.00	0.11	0.24	0.04	0.18
56	0	0	¼	¼	¼	¼	0.00	0.00	0.29	0.15	0.29	0.04	0.23
57	⅕	⅕	⅕	⅕	⅕	0	0.34	0.07	0.16	0.09	0.26	0.00	0.09
58	⅕	⅕	⅕	⅕	0	⅕	0.44	0.11	0.19	0.18	0.00	0.02	0.05
59	⅕	⅕	⅕	0	⅕	⅕	0.35	0.07	0.16	0.00	0.26	0.03	0.13
60	⅕	⅕	0	⅕	⅕	⅕	0.51	0.12	0.00	0.06	0.22	0.03	0.07
61	⅕	0	⅕	⅕	⅕	⅕	0.50	0.00	0.15	0.09	0.19	0.02	0.06
62	0	⅕	⅕	⅕	⅕	⅕	0.00	0.31	0.22	0.09	0.23	0.04	0.12
63	⅙	⅙	⅙	⅙	⅙	⅙	0.36	0.08	0.16	0.10	0.23	0.02	0.05
64	7/12	1/12	1/12	1/12	1/12	1/12	0.55	0.04	0.10	0.09	0.17	0.01	0.04
65	1/12	7/12	1/12	1/12	1/12	1/12	0.22	0.35	0.11	0.08	0.15	0.02	0.07

Mix	PR	C	P	WC	RC	SC	PR	C	P	WC	RC	SC	W
66	1/12	1/12	7/12	1/12	1/12	1/12	0.33	0.06	0.28	0.09	0.16	0.02	0.06
67	1/12	1/12	1/12	7/12	1/12	1/12	0.38	0.11	0.13	0.14	0.14	0.02	0.08
68	1/12	1/12	1/12	1/12	7/12	1/12	0.34	0.08	0.09	0.05	0.35	0.02	0.07
69	1/12	1/12	1/12	1/12	1/12	7/12	0.43	0.12	0.11	0.09	0.12	0.06	0.07

Weighted average proportions of sown species and weed in yield with the yield as the weight.

Table 4.13. Annual DM production dynamics of monocultures and selected swards with sown and VWC proportion from a multi-species mixture experiment at Lincoln University, Canterbury.

Sward type	2018/2019				2019/2020				2020/2021			
	Herbage yield [DM t/ha]	Sown	VWC	Unsown	Herbage yield [DM t/ha]	Sown	VWC	Unsown	Herbage yield [DM t/ha]	Sown	VWC	Unsown
PR	13.32 ± 0.66	0.80	0.14	0.19	12.45 ± 1.05	0.83	0.13	0.17	9.58 ± 1.05	0.98	0.00	0.02
C	9.51 ± 0.52	0.47	0.16	0.53	10.60 ± 0.93	0.83	0.10	0.17	7.80 ± 0.73	0.96	0.02	0.03
P	12.75 ± 0.50	0.66	0.15	0.33	11.14 ± 0.41	0.64	0.19	0.36	11.10 ± 0.99	0.17	0.26	0.83
WC	11.34 ± 0.24	0.57	-	0.43	10.37 ± 0.97	0.56	-	0.44	10.93 ± 0.90	0.31	-	0.69
RC	12.89 ± 0.87	0.74	0.06	0.25	12.87 ± 0.16	0.56	0.14	0.44	12.68 ± 1.25	0.51	0.15	0.49
SC	8.60 ± 0.61	0.34	0.12	0.66	11.14 ± 0.76	0.11	0.01	0.59	12.01 ± 1.13	0.05	0.10	0.94
PR*WC	**11.86 ± 0.48**	**0.91**	**-**	**0.09**	**12.16 ± 1.38**	**0.96**	**-**	**0.04**	**15.13 ± 2.75**	**0.95**	**-**	**0.05**
PR*RC	**14.25 ± 1.09**	**0.89**	**0.03**	**0.11**	**13.69 ± 0.97**	**0.92**	**0.04**	**0.08**	**15.87 ± 1.54**	**0.88**	**0.08**	**0.12**
PR*WC*RC	15.49 ± 0.54	0.92	-	0.08	13.08 ± 1.54	0.96	-	0.03	14.23 ± 1.39	0.96	-	0.04
Binary mix	**12.10 ± 0.57**	**0.74**	**0.07**	**0.26**	**12.43 ± 0.43**	**0.79**	**0.07**	**0.21**	**12.25 ± 0.09**	**0.74**	**0.08**	**0.26**
Ternary mix	12.91 ± 0.30	0.82	0.03	0.18	12.57 ± 0.42	0.89	0.03	0.11	12.50 ± 0.87	0.85	0.05	0.15
Four species mixture	13.38 ± 0.41	0.86	0.02	0.14	13.39 ± 0.46	0.93	0.02	0.07	13.45 ± 0.59	0.90	0.04	0.10
Five species mixture	14.21 ± 0.27	0.90	0.01	0.10	13.74 ± 0.59	0.96	0.01	0.04	14.56 ± 0.63	0.92	0.02	0.08
Centroid mixture	13.46 ± 1.10	0.94	-	0.06	13.80 ± 0.83	0.98	-	0.01	15.09 ± 0.60	0.97	-	0.03
Six species mixture	13.70 ± 0.39	0.90	-	0.10	13.80 ± 0.45	0.97	-	0.03	15.14 ± 1.08	0.98	-	0.02

Chapter 5

Species composition dynamics of diverse species mixed pasture swards

5.1 Introduction

Improved pasture swards are expected to efficiently utilise the above and below-ground growth resources, reducing competition, and maintaining the sown species composition. Maintaining the species proportion in a sward is one key to pasture production practices. Differences in the growth rate of species in a mixture is the main cause of the shifting the botanical composition proportion. The primary physiological process is the relative growth of species to the initial biomass/residual biomass. Growth is defined as an irreversible increase in size accompanied by quantitative changes in biomass (Hay and Porter, 2006). These changes in biomass can be quantified through growth analysis, a widely used tool in plant physiology and ecology (Tilman, 1988). This analysis can be used in comparative studies of plant or pasture sward growth (Pommerening and Grabarnik, 2019) based on time or initial dry weight. Earlier research on quantifying plant/crop growth analysis was based on growth rate over time. This is a standard measure of the growth efficiency of plants. However, plant growth and development are considered a combined effect of genotype (G) and environmental (E) interactions (Chapman et al., 2011). Growth rate differences of associated species can unbalance species composition in the mixture. Changes in species composition can occur in a permanent pasture among perennial species and co-occurring species. Some species may become dominant at the expense of other species, and sward diversity may be reduced.

Shifting the botanical composition in a mixed pasture is generally observed in grasses and legume mix swards. Grasses have a competitive characteristic over legumes and dominate in the swards. Both functional group species must be balanced to maintain sward productivity (Haynes, 1980). Unstable botanical composition over time is one of the challenging tasks pasture production perspectives. Several factors influence pasture performance or compositional changes. The competitive as a relative growth rate relationship is one of the main species-specific characteristics.

5.2 Relative Growth Rate and Relative Growth Rate Differences (RGRD)

Bio-productivity of pasture ecosystems in pastoral farming may be related to: (1) pasture biomass production - increased organic matter and energy content, and (2) size of the assimilation sward system – leaf area, which can include leaf protein and chlorophyll content. Assessing biomass productivity may help select species for seed mixture formulation for higher biomass production.

Among various growth analyses, the Relative Growth Rate (Keating et al.) measures the accumulation rate of new dry biomass per unit of existing dry biomass and is mainly used to quantify the species composition shifting dynamics in a mixed species sward. RGR is a powerful tool in comparative studies of the growth performances of plants (Pommerening and Muszta, 2015). Growth differences between species are the outcome of the competition, and could be defined as a function of competition rather than the initial size (Mac Donald and Weetman, 1993). In pastoral farming, maintaining the component species proportion in the sward is more important than the total biomass yield to maintain herbage quality. The growth differences determine the balance between species in species mixed swards (Woledge, 1988). The concept was first introduced by Blackman (1919), representing the rate of increase in biomass over time or relative to initial biomass as an outcome of the competition in a plant population. It positively correlates with species' resource acquisition and space occupation (Lambers et al., 2008).

Mixed swards community composition dynamics can be examined in several ways. These include relative growth changes, the percentage contribution of each species to the total biomass (Newton et al., 1994; Schenk et al., 1997), or the percentage of total count from point quadrat data. Connolly and Wayne (2005) proposed the RGRD method to study changes in community structure in artificial communities. It can be applied to diverse species mix swards. The Relative Growth Rate (Keating et al.) and Relative Growth Rate Difference (RGRD) are valuable indices to assess community composition changes based on the growth rate of component species. The potential applications of RGR are to (1) compare the growth rate of two species (Keating et al.) to determine which species dominates over time (species balance/compositional change) and. (2) compare the biomass production of monocultures or mixtures over a period (net positive or negative relative growth rate values indicating mixture performance).

The typical relative growth calculation based on the final relative to the initial proportion of a species is presented in Equation 5.1.

Equation 5.1 Calculation of Relative Growth Rate (Keating et al.)

RGR = ln (1/w X W) X 1/dt

w – initial biomass, W – Final biomass, dt – time duration.

The comparison of the changes in species composition for the two species is related to differences in RGRs. The two species are expressed as composition to total biomass at the start and end of the experiment as (y_1/y, y_2/y) and (Y_1/Y, Y_2/Y), respectively. Differences are directly related to the compositional changes, termed relative growth rate difference (RGRD).

y_1 and y_2 are the initial biomass of species 1 and 2; y- initial total biomass.

Y_1 and Y_2 are pre-grazing (Final) biomass at the end of growth cycles; Y- pre-grazing total biomass.

Equation 5.2 Relative Growth Rate Differences

$RGRD = \ln (Y_1/Y \div y_1/y) – \ln (Y_2/Y \div y_2/y) = (RGR_1 - RGR_2)$

$RGRD = (RGR_1 - RGR_2)/t$

RGR or RGRD can be modelled to the initial biomass proportion or environmental condition in each comparison period (1-2 and 2-3 years). If the average relative growth over several re-growth cycles is the same for both species in a unit area over time, there will be no change in composition. In a binary mixture combination, an RGRD value higher than zero (RGRD > 0) indicates the first species gains an advantage; if the value is less than zero (RGRD < 0), the second species has gained at the expense of the first.

5.2.1 Relative Growth Rate Difference (RGRD) modelling

The relative growth rate differences (RGRD) between species as a pasture response can be modelled on the initial condition (initial biomass or abundance), on the inherent relative growth rate of species or on the environmental factors (Connolly et al., 2001), temperature or light. The modelling of RGR or RGRD is useful for understanding pasture plant community dynamics (Suter et al., 2007). The typical model is given in Equation 5.3.

Equation 5.3 Calculation of Relative Growth Rate Differences

$RGRD = bo + b_1X_1 + b_2X_2 + b_3T + b_4PARi + \epsilon$

Where b_1 and b_2 coefficient values -measure the effects of changing the initial green biomass (residual leaf area) of the component species X_1 and X_2 on the differences in average RGRD; b3 and b4 measure the effect of changing accumulated temperature (Tt) and intercepted photosynthetically active radiation (PAR_i) on RGRD.

b_1 / b_2 are called species influential coefficient values, which indicate the intraspecific effects on their own RGR, or comparing them with other species is an interspecific effect on RGRD. Positive values indicate that increasing the species in the initial community (abundance) for initial seed biomass will enhance the differences in RGR in favour of that species, and composition moves positively towards that species. A negative value indicates the opposite trend. The coefficient values b_3 and b_4 represent the environmental variable (independent) that influences RGRD. A positive environmental coefficient value indicates that the respective environmental factor increases the RGRD. A zero-coefficient value of regression implies that the RGR is not affected by the initial composition or exposed environmental variable. In a multi-species hypothetical system, in binary mixture combination, the relative growth rate of species may be altered by intraspecific and interspecific density dependence pasture properties. It implies that the relative abundance and initial biomass can positively or negatively affect the RGR. The combination of these effects stabilizes the pasture function in the system (Brophy et al., 2017). The application of RGR for mixture community is derived from the concept that evaluates the overall performance of mixtures (inter-mixture and intra-mixture) for biomass production. The magnitude of component species' negative and positive RGR values in a mixed community implies the mixture's net effect over time. In this experiment, RGRD values of binary mixtures regressed against the RGR of species as a species-specific characteristic and intercepted light energy. The potential drivers of RGRD of mixture components are the inherent RGR of species (Aarssen, 1983), initial biomass (Suter et al., 2010), climatic condition (Van der Putten et al., 2010) and management (Nyfeler et al., 2009a).

5.2.2 Ecological consequences of differences in species growth rate

The differences in RGR in mixture communities results from the inherent RGR of species with the combined effects of available resources in the growing environment (Poorter and Garnier, 1996). The differences in growth may be because of physiological, morphological and anatomical differences (Poorter and Garnier, 1996). Environmental factors such as light, CO_2, temperature, water, and nutrients across the season also affect the growth and development of pasture species. Interspecific variation in relative growth rate also exists in a species mixed pasture sward, For example, grass or legume functional component, C_3 or C_4 photosynthetic pathway. The relative dry mass production per unit of initial dry biomass also differed even when comparing them in similar growing environments (Grime and Hunt, 1975)

The ecological importance of relative growth rate is the classification of species based on growth rate (Grime, 1979), such as slow and fast-growing species. A species with a higher relative growth rate will rapidly increase in size and may occupy a larger space below and above ground and access more growth resources, light, nutrients, and water than the slow-growing species. The higher growth rate species would produce more biomass and supply more feed and may reduce the grazing interval. In contrast to fast-growing species, slow-growing species have less demand for growth resources and produce less biomass even in suitable climatic condition. Due to the habitat-related variation, slow-growth species have less ability to build biomass, photosynthate and minerals in structural materials (Chapin, 1980; Hunt, 1978). The differences in the growth rate of the mixture create an imbalance in botanical composition. A key issue in the ecology and agronomy of diverse species of mixed grassland is the quantification of how individuals of co-occurring species affect and respond to each other and how these interactions affect the structure of plant communities (Connolly et al., 2001; Grace and Tilman, 1990). The community composition dynamics depend on many processes other than the growth rate, such as the formation of new individuals, survival of species, spatial structure and scale (Freckleton and Watkinson, 2000). All these processes contribute to the DM increment and shift species composition in a species mixed sward.

The relative growth rate of pasture species in a mixed community is complex, and these species interact directly with climatic factors and soil fertility status. In this experiment, fertilizer N was limited in the system and was expected to be provided from the biologically fixed N from cooccurring legume functional species. Species with higher relative growth allow them to occupy space rapidly, capture resources, and produce higher biomass than the slow-growing species. The species which show a higher relative growth rate produces higher biomass yield after a certain period of growth, but the biomass production depends on the initial mass or is related to the seed mass in the establishment year. Seed mass determines the size of a seedling at the beginning or any time after germination (Fernández Alés et al., 1993; Marañón and Grubb, 1993). This experiment did not consider the establishment year growth comparison (first-year biomass of species) with the initial seed weight of species. The relative dry matter yield should remain the same for longer to maintain the pasture composition and production stability. A binary combination of legumes and non-legumes would be appropriate when considering no-fertiliser application. The appropriate species combined with a similar relative growth rate in the mixtures will seldom reflect changes in composition and sustain the improved pasture yield.

Generally, interest in maintaining the botanical composition of diverse pastures begins when dry matter production is reduced or quality changes or begins with the infestation and multiplication of weeds exceeding the economic threshold level or earlier. By understanding this, management interventions can be made to improve pasture productivity. Agronomic management practices that begin from seed formulation are associated with the balance of component species in the sward with an almost equal relative growth rate of species. Analysis of permanent pasture component species' relative growth rate over time is necessary to explain the balance in species composition. This experiment continuously tested the botanical composition of a range of pasture mixtures created from commonly used species (PR, C, P, WC, RC, and SC) under sheep grazing and no fertilizer application. The following objectives were assigned to understand the mixture component dynamics and persistence over the years.

Objective 5. To compare the inter and intra mixture productivity (herbage biomass) based on net RGR of component species.

Objective 6. To quantify the botanical composition dynamics of binary mixtures (Relative Growth Rate Differences modelling).

Objective 7. To Identify an optimal component proportion to maintain the botanical composition of ternary mixture (PR*WC*RC- DI modelling for RGRD).

Objective 8. To quantify the effect of RGR as a mixture component species property and intercepted light energy on botanical composition changes of binary mixtures.

5.3 Materials and Methods

Relative growth rate comparison was performed in three different ways:
(1) Monoculture relative growth rate was compared between the two main growth periods to assess the growth rate of species (interspecific and intraspecific growth assessment).

(2) Assessing RGRD -In binary mixtures, quantifying RGR of the first species to the second species

(3) The summation of component species' relative growth rate (net growth rate) was used to compare biomass yielding potential of mixtures between 1-2 years and 2-3 years. Further, average RGRD was modelled to the sown proportion of component species to identify the appropriate species proportion of component species (PR*WC*RC) to minimize the changes in botanical composition of the mixture community.

Relative Growth Rate Difference (RGRD) values of binary mixtures were calculated to assess the changes in species composition (Connolly and Wayne, 2005). Calculations were done according to Equation 5.1 and Equation 5.2. The natural logarithm of the average mixture component yield proportion of the second year to the average botanical composition proportion of the first year gives RGR calculation for 1–2-year comparison. Similarly, the RGR of 2-3 year comparison was calculated for the third year; the natural logarithm of the third-year biomass yield proportion to the average biomass yield proportion of the second year (Brophy et al., 2017; Suter et al., 2007). A minimum value of 0.001 g was added if the mixture did not have dry matter yield for the component species.

RGRD values were regressed against the growth rate of species (Keating et al.), and cumulative intercepted light energy (PARi). The regression prediction identifies the species-specific RGR and intercepted light energy on botanical composition shifting of binary mixtures or RGRD values.

5.4 Results

5.4.1 Botanical composition

Average botanical composition over three years related to the sown seed proportion is given in Table 4.12. The botanical composition proportion of monocultures and species mixed swards was unstable and differed between mixture combinations. In monoculture species, grasses had greater proportion of 0.77 and 0.88 for cocksfoot and perennial ryegrass. The sown proportion of SC, WC, P and RC were 0.19, 0.47, 0.50 and 0.55, respectively (Table 4.12). The unsown proportion across the monoculture species was 0.42 whereas in species mixed swards it was 0.15 (Figure 4.5). The botanical composition proportion of PR*WC, PR*RC and PR*WC*RC over three years was 0.73:0.20, 0.56:0.33 and 055:0.07:0.32, respectively. The composition proportion such as sown species, VWC and weeds for mixture communities are shown in Figure 4.6 and Figure 4.7. The relative growth rate comparison of monocultures over time and, similarly, net relative growth rate of mixture communities gave the intra and inter mixture productivity over time, present the biomass production of sown species.

5.4.2 **The relative growth rate of swards**

Interspecific variation in the relative growth rate of monoculture species were quantified and presented in Table 5.1. The relative growth rate of species presented the rate of dry matter production per unit of initial dry matter or growth efficiency for 1-2- and 2-3-year comparison periods. RGR differed among species ($p<0.05$) and was not consistent over both comparison periods. The values ranged from - 0.346 to 0.393 and - 0.525 to 0.163 to in the first and second comparison periods, respectively. Positive and negative values indicate the biomass increase or decrease relative to the previous year's production. Grasses had higher innate growth potential than clovers. Perennial ryegrass and cocksfoot biomass production rates were 0.001 $tt^{-1}ha^{-1}yr^{-1}$ and 0.39 $tt^{-1}ha^{-1}yr^{-1}$, respectively in the first comparison period. In the second comparison period 0.163 and 0.132 $tt^{-1}ha^{-1}yr^{-1}$. The biomass production rate of RC and SC were negative (– 0.11 and – 0.34 $tt^{-1}ha^{-1}yr^{-1}$) in the first comparison period and positive (0.032 $tt^{-1}ha^{-1}yr^{-1}$) for SC in the second comparison period. The positive and negative relative growth rate value for P indicated that the biomass production rate of the plantain was increased in the 2nd year and reduced in the 3rd year.

Table 5.1 RGR of monoculture species between two comparison period in a multi-species mixture experiment at Lincoln University, Canterbury, New Zealand (2020/21).

1st - 2nd year t t^{-1} $year^{-1}$			2nd – 3rd year t t^{-1} $year^{-1}$		
PR	+ 0.001	b	PR	+ 0.163	a
C	+ 0.393	a	C	+ 0.132	a
P	+ 0.021	b	P	- 0.525	c
WC	+ 0.026	b	WC	- 0.182	b
RC	- 0.115	b	RC	- 0.047	ab
SC	- 0.346	c	SC	+ 0.032	ab

The same letter in a column does not differ significantly in a comparison period. Species notation is given in Chapter 3.3

The relative biomass production (sward biomass) of mixture component species determines the mixture performance. The net RGR (positive and negative) of mixtures was used to compare the mixtures' biomass production efficiency for 1-2 year and 2–3-year comparison periods for determining the inter-mixture and intra-mixture biomass production efficiency.

The mixture's relative growth rate was the net effect of component species' positive and negative RGR (Vile et al., 2006) due to competition (Michalet et al., 2023). Several mixture combinations showed a negative relative growth rate in a 2–3-year comparison (Table 5.2), indicating that, on average, the biomass production efficiency of species mixed swards was reduced in the 3rd year compared to the 2nd year. On average, 75% of mixture combinations showed negative RGR in the second comparison period.

Comparison of intra-mixture biomass production ability of mixed pasture swards showed that 65% of ternary mixtures (13 out of 20), 93% of four species mixtures (14 out of 15), and 83% of five and six species mixtures (5 out of 6) showed a negative growth rate in 2–3-year comparison period. implies that growth and biomass production efficiency decreased in the third year compared 1 -2 years. Among the binary mixtures, RGR for PR*WC and PR*RC in the 1–2-year comparison period were 0.24 and 0.30 $tt^{-1}ha^{-1}yr^{-1}$, respectively. In the second comparison period, PR*WC biomass production trend was -0.03 $tt^{-1}ha^{-1}yr^{-1,}$ and 0.1 $tt^{-1}ha^{-1}yr^{-1}$ for PR*RC. PR*WC*RC ternary mixture maintained a constant biomass production rate of 0.03 $tt^{-1}ha^{-1}yr^{-1}$ in the 2nd and 3rd comparison periods.

Table 5.2 Net relative growth rate of mixtures (summation of the relative growth rate of component species) in a pasture mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Mix	Component species	RGR ($tt^{-1}ha^{-1}$) 1st to 2nd year	RGR ($tt^{-1}ha^{-1}$) 2nd to 3rd year
7	PR*C	0.07	-0.02
8	PR*P	-0.19	0.13
9	PR*WC	0.3	-0.03
10	PR*RC	0.24	0.1
11	PR*SC	0.24	0.05
12	C*P	0.12	0.08
13	C*WC	0.05	-0.2
14	C*RC	0.13	-0.28
15	C*SC	-0.27	-0.18
16	P*WC	0.01	-0.05
17	P*RC	0	0.16
18	P*SC	-0.22	-0.13
19	WC*RC	-0.15	-0.13
20	WC*SC	0.16	-0.08
21	RC*SC	0.06	0.06
22	PR*C*P	0.1	-0.03
23	PR*C*WC	0.01	0.12
24	PR*C*RC	0.1	-0.01
25	PR*C*SC	0.08	-0.07
26	PR*P*WC	-0.13	-0.15
27	PR*P*RC	0.08	0
28	PR*P*SC	0.39	0.13
29	PR*WC*RC	-0.03	-0.03
30	PR*WC*SC	0.02	-0.09
31	PR*RC*SC	0.35	-0.04
32	C*P*WC	0.2	-0.02
33	C*P*RC	0.09	-0.12
34	C*P*SC	0.32	0.04
35	C*WC*RC	0.08	0
36	C*WC*SC	0.11	0.01
37	C*RC*SC	0.11	-0.09
38	P*WC*RC	-0.1	-0.14
39	P*WC*SC	0.02	-0.53
40	P*RC*SC	-0.06	-0.13
41	WC*RC*SC	-0.05	0.01
42	PR*C*P*WC	0.15	-0.09
43	PR*C*P*RC	0.13	-0.07
44	PR*C*P*SC	0.08	-0.08
45	PR*C*WC*RC	0.14	-0.03
46	PR*C*WC*SC	-0.01	0.05
47	PR*C*RC*SC	0	-0.01
48	PR*P*WC*RC	0.08	-0.04
49	PR*P*WC*SC	0	-0.03
50	PR*P*RC*SC	0.03	-0.18
51	PR*WC*RC*SC	0.01	-0.02
52	C*P*WC*RC	0	-0.02
53	C*P*WC*SC	0.26	-0.16
54	C*P*RC*SC	0.15	-0.04
55	C*WC*RC*SC	0.19	-0.04

Species notation is given in Section 3.3.

Cont. Table 5.2

Mix	Component species	RGR ($tt^{-1}ha^{-1}$) 1st to 2nd year	RGR ($tt^{-1}ha^{-1}$) 2nd to 3rd year
56	P*WC*RC*SC	0.21	-0.01
57	PR*C*P*WC*RC	0.02	-0.1
58	PR*C*P*WC*SC	0.14	-0.08
59	PR*C*P*RC*SC	0.04	-0.02
60	PR*C*WC*RC*SC	0.06	-0.08
61	PR*P*WC*RC*SC	-0.11	-0.05
62	C*P*WC*RC*SC	0.07	0.01
63	PR*C*P*WC*RC*SC	0.07	-0.03
64	PR*C*P*WC*RC*SC	0.19	-0.06
65	PR*C*P*WC*RC*SC	0.06	0
66	PR*C*P*WC*RC*SC	0.09	-0.02
67	PR*C*P*WC*RC*SC	0.16	-0.02
68	PR*C*P*WC*RC*SC	0.09	-0.03
69	PR*C*P*WC*RC*SC	0.18	0.01

Species notation is given in Section 3.3.

5.4.3 **Relative Growth Rate Difference analysis for binary mixtures**

RGRD values of binary mixtures for the first and second comparison period are given in Table 5.3 and Table 5.4. Positive value indicate that the first species dominates in the mixture relevant to the second species or reduces the associated second component species relevant to the first species in the community. The negative value expresses the opposite trend. In the first comparison period, positive and negative RGRD values showed dominating or suppressing mixture component dynamics relevant to the first species in the binary notation. RGRD values differed from - 0.021 for P*WC to 1 for PR*WC in the first comparison period. In PR*WC binary mixture, PR dominated in the second year while the WC population diminished. RGRD value was more than one (RGRD > 1), indicating that PR dominated in 1st comparison period. Plantain showed a positive RGR in binary combinations except for C*P in the 1st comparison period. RGR of SC was negative in all binary combination, while RC and WC showed both positive and negative RGRs when associated with other component species (Table 5.3). The PR*RC, PR*C, and PR*P mixtures showed relative growth rate difference values of 0.07,-0.079 and 0.021, respectively indicating a lower rate of compositional changes.

Table 5.3 RGR of component species of binary mixtures and relative growth rate difference (RGRD) value between component species for the first comparison period (1-2 years) in a pasture species mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Binary mixtures	**Growth Period 1 (1-2 years) 2018/2019 to 2019/2020**						
Species	**PR**	**C**	**P**	**WC**	**RC**	**SC**	**RGRD**
PR*RC	0.078				0.005		- 0.073
PR*SC	0.127					- 0.316	0.443
C*P		0.498	- 0.200				0.689
C*WC		0.402		-0.165			0.567
C*RC		0.380			-0.197		0.577
C*SC		0.507				- 0.385	0.892
P*WC			0.165	0.037			- 0.205
P*RC			0.040		0.082		- 0.042
P-SC			0.031			- 0.303	0.334
WC*RC				0.120	0.047		0.235
WC*SC				0.154		- 0.371	0.525
RC*SC					- 0.072	- 0.235	0.322
PR*C	0.068	0.147					- 0.079
PR*P	0.049		0.027				0.021
PR*WC	0.148			-0.940			1.000

Species notation is given in Section 3.3.

In the second comparison period, the relative growth differences between species in binary combinations ranged from – 0.018 in RC*SC to 0.645 in C*WC. RGRD values in the 2–3-year comparison period (2nd) was negative in several binary mixture combinations (Table 5.4). Out of 15 mixtures, 9 showed negative relative growth rate. PR*RC binary mixture composition changes was different from the first comparison period. The RGR of PR was negative and RC dominated (+0.12) in the mixture giving a negative RGRD value (-0.256). C continuously dominated with associated component species in the 1st and 2nd comparison period (Table 5.3 and Table 5.4). RGR of SC was negative in the first and second comparison periods.

Table 5.4 RGR of component species of binary mixtures and Relative Growth Rate Difference (RGRD) between component species for the 2nd comparison period (2nd to 3rd year) in a pasture species mixture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Binary mixtures	**Growth Period 2 (2-3 years) 2019/2020 to 2020/2021**						
Species	**PR**	**C**	**P**	**WC**	**RC**	**SC**	**RGRD**
PR*RC	- 0.137				0.119		-0.256
PR*SC	0.135					0	0.135
C*P		0.271	- 0.304				0.575
C*WC		0.674		0.029			0.645
C*RC		0.031			0.019		0.012
C*SC		0.083				- 0.008	0.091
P*WC			- 0.367	0.169			- 0.536
P*RC			- 0.366		0.082		- 0.448
P*SC			- 0.172			- 0.008	- 0.164
WC*RC				- 0.096	0.047		- 0.143
WC*SC				- 0.125		- 0.008	- 0.117
RC*SC					- 0.072	- 0.054	- 0.018
PR*C	- 0.102	0.229					- 0.331
PR*P	0.083		- 0.209				0.292
PR*WC	- 0.151			0.159			-0.310

Species notation is given in Section 3.3

5.4.4 Botanical composition proportion of mixtures

The botanical composition was calculated based on the DM production of sown species to the total biomass, considered as the proportion of botanical composition of the sward. Sown proportion of PR*WC and PR*RC binary mixtures ranged from 0.88 to 0.86 over three years, respectively (Table 5.5). Binary mixtures maintained a higher sown proportion in the grass-perennial legume and grass-herb mixture. Legume-legume and the legume-herb mixture maintained a lower sown proportion. PR association with WC and RC in binary combination showed fluctuating component species, ranging from 0.49 to 0.83 PR and 0.12 to 0.41 clovers (WC and RC). These mixtures showed a significant intermittent interaction between component species for biomass production over 23 re-growth cycles (Appendix I).

Table 5.5 Total sown species proportion dynamics of binary mixtures in multi-species pasture experiment from 2018 to 2021 at Lincoln University, Canterbury, New Zealand.

Mixture combination	**Sown yield proportion of binary mixtures**			**sown Yield**
	2018/2019	**2019/2020**	**2020/2021**	**(t/ha)**
PR*C	0.69	0.88	0.99	1.54 ± 0.10
PR*P	**0.85**	**0.91**	**0.80**	1.72 ± 0.18
PR*WC	**0.92**	**0.96**	**0.95**	1.70 ± 0.14
PR*RC	**0.88**	**0.92**	**0.88**	1.90 ± 0.11
PR*SC	0.87	0.85	0.98	1.71 ± 0.08
C*P	0.65	0.85	0.81	1.54 ± 0.12
C*WC	0.69	0.83	0.94	1.50 ± 0.09
C*RC	0.71	0.86	0.89	1.94 ± 0.09
C*SC	0.59	0.85	0.90	1.31 ± 0.11
P*WC	0.78	0.78	0.63	1.51 ± 0.08
P*RC	0.72	0.80	0.59	1.55 ± 0.15
P*SC	0.74	0.57	0.26	1.41 ± 0.06
WC*RC	0.67	0.60	0.50	1.62 ± 0.09
WC*SC	0.65	0.56	0.40	1.41 ± 0.12
RC*SC	0.64	0.53	0.41	1.56 ± 0.11
Centroid Mixture	0.94	0.98	0.97	1.84 ± 0.08

Species notation is given in Chapter 3.3

On average, out of 15 binary mixtures, 11 had more than 80% of component species in the second year, except for the mixture containing sub-clover. In the third year, 10 binary mixtures had a minimum species proportion of 12% (Table 5.6). The annual growth habit of SC provided less contribution in the second and third years, allowing the room for more weeds and reducing the sown biomass yield proportion.

Table 5.6 Sown component species proportion dynamics of binary mixtures in a multi-species mixture experiment with the number of significant interactions for DM yield over the 23 regrowth cycles at Lincoln University, Canterbury, New Zealand.

Binary mixtures	Sown component species proportion dynamics			Number of significant interactions
	2018/2019	2019/2020	2020/2021	
PR:C	0.67 : 0.02	0.74 : 0.14	0.64 : 0.35	5
PR:P	0.51 : 0.33	0.56 : 0.36	0.64 : 0.16	3
PR:WC	0.68 : 0.20	0.83 : 0.12	0.68 : 0.27	7
PR:RC	0.55 : 0.30	0.63 : 0.29	0.49 : 0.41	9
PR:SC	0.72 : 0.15	0.84 : 0.00	0.98 : 0.00	11
C:P	0.07 : 0.57	0.45 : 0.38	0.71 : 0.12	1
C:WC	0.30 : 0.32	0.68 : 0.16	0.75 : 0.19	6
C:RC	0.18 : 0.46	0.53 : 0.11	0.56 : 0.35	11
C:SC	0.36 : 0.22	0.84 : 0.01	0.92 : 0.00	6
P:WC	0.54 : 0.18	0.56 : 0.22	0.22 : 0.38	3
P:RC	0.38 : 0.30	0.42 : 0.09	0.12 : 0.47	1
P:SC	0.54 : 0.19	0.57 : 0.01	0.24 : 0.00	5
WC:RC	0.18 : 0.41	0.29 : 0.08	0.20 : 0.34	3
WC:SC	0.39 : 0.27	0.54 : 0.02	0.42 : 0.42	5
RC:SC	0.40 : 0.23	0.08 : 0.04	0.21 : 0.04	3
SE	0.32 : 0.22	0.42 : 0.27	0.54 : 0.34	

Species notation is given in Section 3.3.

The component species proportion of ternary mixture is shown in Table 5.7. Overall biomass yield of component proportion showed that grasses with one or two perennial clovers were effective mixture components that maintained the composition. The highlighted mixture combinations in Table 5.7 maintained the component proportion. The mixture consisting of PR*WC*RC at an equal proportion (0.33) maintained more than 0.30 fraction of clovers (WC/RC) of sward herbage biomass, even fluctuating individual components, and had 0.05 and 0.06 fraction of WC in the second and third years. SC was an effective component in the establishment year and then continuously reduced. In C based ternary mixtures, the proportion of C was lower in first year and progressively increased over the second and third years.

Table 5.7 Sown component species proportion dynamics of ternary mixtures and the number of significant interactions for DM yield over the 23 regrowth cycles in a pasture mixture experiment at Lincoln University, Canterbury, New Zealand.

Ternary mixtures	**Sown component species proportion dynamics**			**Number of significant interactions**
	2018/2019	**2019/2020**	**2020/2021**	
PR:C:P	0.49 : 0.02 : 0.26	0.45 : 0.08 : 0.38	0.43 : 0.29 : 0.11	2
PR:C:WC	0.60 : 0.02 : 0.25	0.64 : 0.09 : 0.17	0.40 : 0.26 : 0.30	0
PR:C:RC	0.53 : 0.02 : 0.31	0.61 : 0.07 : 0.26	0.35 : 0.20 : 0.36	0
PR:C:SC	0.65 : 0.03 : 0.12	0.71 : 0.16 : 0.01	0.51 : 0.45 : 0.00	0
PR:P:WC	0.39 : 0.35 : 0.14	0.53 : 0.34 : 0.11	0.72 : 0.12 : 0.15	2
PR:P:RC	0.39 : 0.22 : 0.26	0.43 : 0.26 : 0.26	0.45 : 0.08 : 0.37	0
PR:P:SC	0.51 : 0.26 : 0.11	0.51 : 0.33 : 0.00	0.55 : 0.15 : 0.00	1
PR:WC:RC	**0.50 : 0.10 : 0.28**	**0.64 : 0.05 : 0.26**	**0.50 : 0.06 : 0.40**	2
PR:WC:SC	0.67 : 0.11 : 0.12	0.76 : 0.19 : 0.01	0.64 : 0.27 : 0.00	0
PR:RC:SC	0.50 : 0.23 : 0.12	0.72 : 0.21 : 0.01	0.51 : 0.35 : 0.00	2
C:P:WC	0.05 : 0.45 : 0.22	0.41 : 0.40 : 0.13	0.54 : 0.15 : 0.22	0
C:P:RC	0.04 : 0.37 : 0.31	0.29 : 0.31 : 0.26	0.42 : 0.10 : 0.37	0
C:P:SC	0.11 : 0.49 : 0.15	0.56 : 0.30 : 0.01	0.71 : 0.08 : 0.00	2
C:WC:RC	0.16 : 0.12 : 0.35	0.57 : 0.09 : 0.24	0.56 : 0.07 : 0.31	1
C:WC:SC	0.24 : 0.26 : 0.17	0.74 : 0.15 : 0.00	0.65 : 0.24 : 0.00	0
C:RC:SC	0.14 : 0.35 : 0.18	0.51 : 0.32 : 0.01	0.47 : 0.39 : 0.00	1
P:WC:RC	0.40 : 0.09 : 0.26	0.46 : 0.08 : 0.31	0.14 : 0.11 : 0.53	1
P:WC:SC	0.50 : 0.13 : 0.16	0.54 : 0.23 : 0.01	0.22 : 0.42 : 0.00	3
P:RC:SC	0.42 : 0.21 : 0.13	0.40 : 0.35 : 0.01	0.16 : 0.49 : 0.00	1
WC:RC:SC	0.15 : 0.35 : 0.17	0.27 : 0.36 : 0.02	0.28 : 0.38 : 0.00	0
SE	0.03 : 0.02 : 0.02	0.04 : 0.03 : 0.01	0.05 : 0.04 : 0.02	

Species notation is given in Section 3.3. PR*WC*RC (0.33:0.33:0.33) is highlighted.

Table 5.8 Sown component species proportion of four species mixture in a diverse pasture species mixture experiment for the 2019/20 and 2020/21 production year at Lincoln University, Canterbury, New Zealand.

Swards	Sown component species proportion dynamics	
	2019/2020	2020/2021
PR:C:P: WC	**0.40 : 0.11 : 0.37 : 0.10**	**0.41 : 0.29 : 0.06 : 0.20**
PR:C:P:RC	**0.41 : 0.05 : 0.27 : 0.21**	**0.31 : 0.14 : 0.07 : 0.38**
PR:C:P:SC	0.56 : 0.08 : 0.25 : 0.00	0.44 : 0.28 : 0.07 : 0.00
PR:C:WC:RC	**0.47 : 0.14 : 0.06 : 0.31**	**0.35 : 0.23 : 0.10 : 0.27**
PR:C:WC:SC	0.70 : 0.13 : 0.13 : 0.00	0.41 : 0.35 : 0.23 : 0.00
PR:C:RC:SC	0.58 : 0.05 : 0.28 : 0.00	0.36 : 0.18 : 0.34 : 0.00
PR:P:WC:RC	**0.42 : 0.24 : 0.05 : 0.27**	**0.45 : 0.06 : 0.08 : 0.38**
PR:P:WC:SC	0.50 : 0.28 : 0.18 : 0.00	0.61 : 0.09 : 0.26 : 0.00
PR:P:RC:SC	0.52 : 0.17 : 0.22 : 0.01	0.48 : 0.05 : 0.38 : 0.00
PR:WC:RC:SC	0.62 : 0.08 : 0.25 : 0.01	0.45 : 0.08 : 0.41 : 0.00
C:P:WC:RC	**0.30 : 0.30 : 0.07 : 0.25**	**0.46 : 0.04 : 0.07 : 0.33**
C:P:WC:SC	0.46 : 0.33 : 0.14 : 0.00	0.67 : 0.09 : 0.18 : 0.00
C:P:RC:SC	0.39 : 0.21 : 0.25 : 0.01	0.51 : 0.05 : 0.33 : 0.00
C:WC:RC:SC	0.58 : 0.10 : 0.21 : 0.01	0.53 : 0.11 : 0.23 : 0.00
P:WC:RC:SC	0.37 : 0.14 : 0.30 : 0.01	0.15 : 0.20 : 0.38 : 0.00
SE	0.00 : 0.01 : 0.03 : 0.00	0.03 : 0.00 : 0.06 : 0.00

Note: Mixtures containing more than 5 per cent of sown species biomass were highlighted in the second and third years of production with a mean, standard error (SE) ratio of component species.

Component species such as SC and P proportions were reduced in the 3rd year after establishment while WC fluctuated. Grass-clover-herb-based four species mixture subclover in the mixture showed an ability to self-regulates its component species to form a simple mixture at 2 to 3 species level. The highlighted mixtures maintained a minimum component species proportion of 0.05.

5.4.5 PR*WC*RC mixture proportion over three years

Optimization of PR*WC*RC ternary mixture for improved pasture responses was discussed in Section 4.4.1 These mixture component species dynamics over three years were presented in a ternary diagram Figure 5.1. The diagram is conveniently represented moving component proportions in a triangular diagram where each side corresponds to an individual or two-sided-binary or three-sided-ternary dynamic point. The first, second and third year modelled component proportions were 0.57:0.11:0.32, 0.67:0.06:0.27 and 0.52:0.06:0.42, respectively. The PR*WC*RC mixture component species proportion was moving closer to each other annually, and the more weightage of component proportion from PR and RC showed oscillating dynamics.

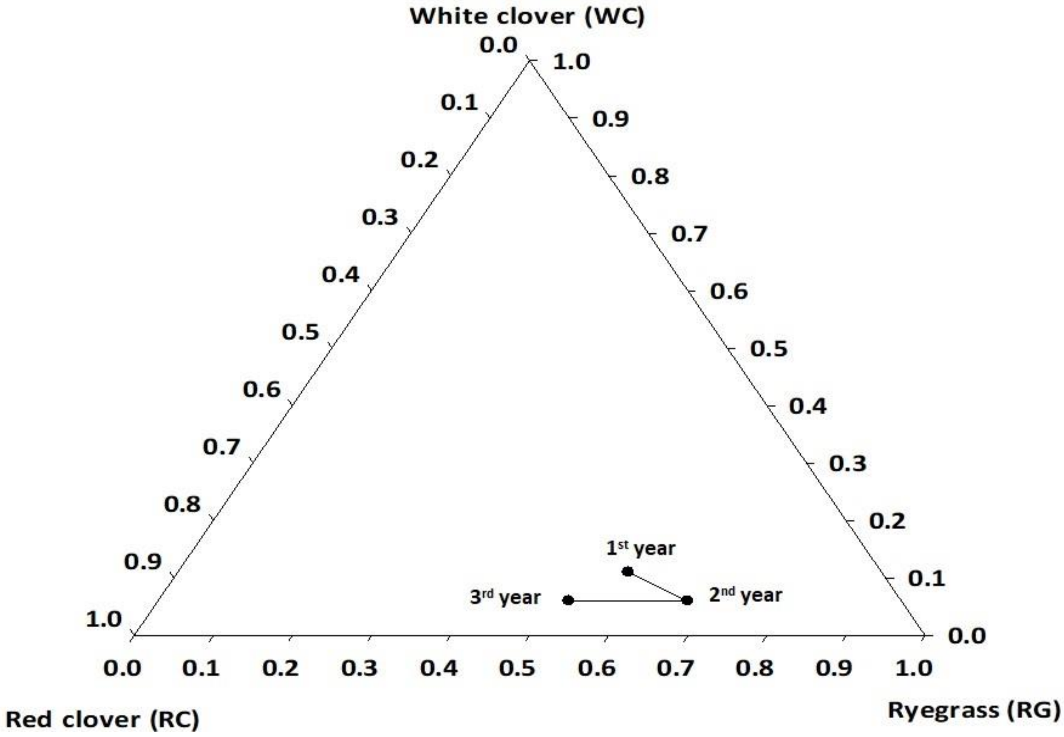


Figure 5.1 The sown proportion composition dynamics of PR:WC:RC mixture over the years from 2018/19 to 2020/21 at Lincoln University, Canterbury, New Zealand.

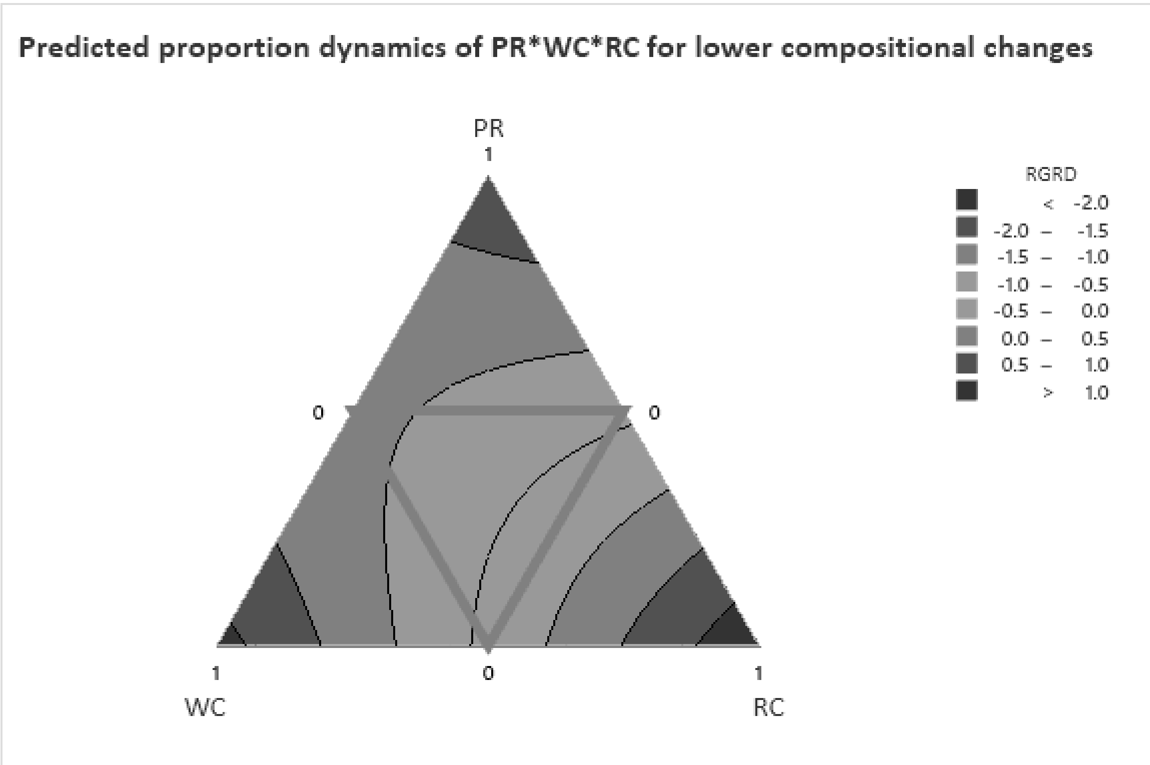


Figure 5.2 Component species proportion of PR*WC*RC for lower RGRD over three years at Lincoln University, Canterbury, New Zealand (2018-2021).

Figure 5.2 represents the RGRD value of the PR*WC*RC ternary mixture to the different sown proportions. The marked triangle in the middle of the ternary plot shows a lower RGRD, which was at the equal component species proportion in the mixture. Based on Figure 5.2, an unequal or unbalanced mixture of species leads to a higher positive or negative RGRD value, indicating the compositional changes of the mixture(Appendix G).

5.4.6 Sown species proportion dynamics of centroid mixture

Centroid mixture represents the equal relative abundance of the component species in the experiment, a typical species-rich and high-evenness (1) mixture. Figure 5.3 shows the component species proportion dynamics over three years. No significant interactions between component species were observed (Chapter 4), model analysis did not quantify the strength of interaction. Over three years, the average sown proportion of centroid mixture was more than 94%, and mixture component species fluctuated drastically. Over three years, the biomass composition proportion of PR in the centroid mixture was 0.50 (0.52 to 0.67), and WC showed a reducing trend from 0.11 to 0.06 from the first to third year. RC component biomass proportions were 0.32, 0.27 and 0.42 each year from establishment. C showed a continuously increasing proportion from 0.01 to 0.17 in 3rd year, whereas the P proportion reduced to 0.07 in the 3rd year.

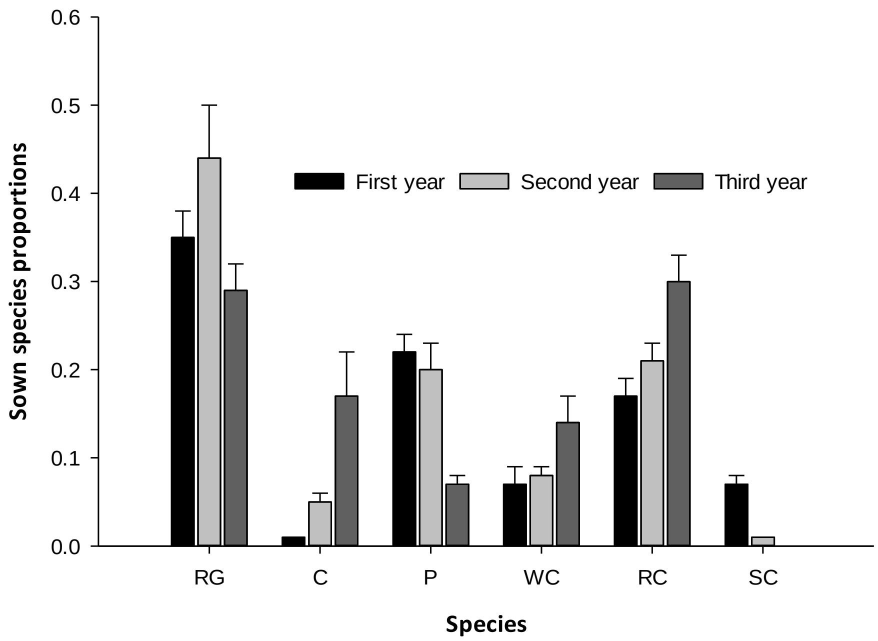


Figure 5.3 Component species dynamics of centroid mixture (even mixture) in a pasture diversity experiment at Lincoln University, Canterbury, New Zealand (2018-2021).

5.4.7 Quantifying the effect of light and species specific relative growth rate on RGRD of binary mixtures

Equation 5.4 Regression prediction equation for quantifying the species and light effects on RGRD.

RGRD = – 1.193 + 2.83 PR + 0.36 C + 0.16 P– 1.24 WC – 1.85 RC – 1.01 SC + 0.01 Cu PARi

R (sq) = 87.6%, R (sq-adj) = 85.9%, P = 0.01

The regression prediction model identified that the RGR of component species (as a species effect) and intercepted PAR energy, significantly affected overall growth differences of the binary mixture combinations. The data fitted well with the regression (87.6%) equation. The component species showed both a positive and negative impact on growth rate differences. The higher growth rate coefficient value for PR (2.83) indicates that PR exerted a positive significant effect on compositional changes compared to C (0.36) or plantain (0.16). The legume component negatively affected the mixture, meaning it also exerted influence on compositional change. The magnitude of the effect (coefficient value) ranged from 1.0 for SC to 1.85 for red clover. Intercepted light energy had less effect (coefficient value-0.01) on RGRD than the species-specific relative growth rate.

5.5 Discussion

This discussion is mainly based on the aim of the chapter, to assess the botanical composition dynamics of swards, which is the result of the competitive success of the component species in a species mixed pasture sward. The RGR values were used to compare the intra and inter-mixture biomass production potential, which is one of the indices to evaluate the sown species biomass production potential, not for assessing the botanical composition. The changes in botanical composition in each time interval were quantified by comparing the Relative Growth Rate Difference (RGRD) values between component species of binary mixtures. RGRD value indicated the rate of change and direction of change in the mixture community, relevant to each other – binary mixture. This is a useful indicator of pasture stability, affecting the system productivity.

Changes in the botanical composition of mixtures that took place over time are unavoidable in multi-species pasture swards. Comparing monoculture species between the two main periods showed that grass functional components have a positive RGR value, meaning grass in monocultures dominated over time. Plantain showed an increasing trend in the first comparison period (1st to 2nd year) and reduced in the second and third periods (2nd to 3rd year). Perennial clovers such as WC and RC in monoculture communities showed a different pattern. RC biomass was reduced continuously for both comparison periods. However, WC increased in the first comparison period (1^{st} to 2^{nd} year) and reduced in the second comparison period (2^{nd} to 3^{rd} year). The annual comparison of the net relative growth rate of each species in the mixture was conducted both within individual mixture and between different mixtures. This comparison was aimed at quantifying the efficiency of each mixture in producing biomass both internally (intra-mixture efficiency) and in comparing with other mixtures (inter-mixture efficiency) as described by Pommerening and Muszta (2015). The mixtures showed a positive and negative growth rate in 1-2 year and 2–3 year comparisons. Compared with the 1-2 year (1^{st}) comparison period, the biomass production efficiency of mixtures was reduced in the 2^{nd} to 3^{rd} year, accounting for 75% of mixture combinations. PR*WC and PR*RC produced higher biomass in the second year compared with the first year (PRWC = 0.30, PRRC = 0.24 $tt^{-1}ha^{-1}$). Third-year biomass production was lower than the second year for PR*WC (PR*WC = -0.03 $tt^{-1}ha^{-1}$) and higher for PR*RC (PR*RC = 0.10 $tt^{-1}ha^{-1}$).

Relative Growth Rate Difference values (RGRD) of binary mixtures showed positive and negative values depending on associated functional component species. In the first comparison period (1-2 years), PR dominated in the binary mixture PR*RC. In the second comparison period (2-3 years), PR showed a negative RGR, and RC showed a positive RGR. RGRD values of PR*WC showed that PR dominated in the first comparison period at the expense of WC, whereas in the second comparison period, RGR of WC increased and RGR of PR was reduced (2^{nd} to 3^{rd} year).

Identified mixture communities that maintain the botanical composition proportions were PR*WC, P*WC, PR*P*RC, PR*C*RC, PR*C*WC and C*WC*RC. The mixture potential to suppress the weeds was one of the agronomic potentials for the optimisation of component species. The unsown species proportion in the mixture communities was lower when compared with monocultures and continuously reduced with an increasing number of species in the mixture (richness).

In this experiment, sown species proportion was increased with species richness. In general, plant diversity enhanced the total biomass of sown species (Finn et al., 2013b). A pasture study across 28 sites in Europe also showed that unsown species were lower in species mixed pasture communities than in monocultures (Kirwan et al., 2007). In this experiment, the average sown proportion ranged from 0.76 in the binary mixture to 0.95 in the six-species combinations (Table 4.13). An optimal mixture forming component species (PR*WC*RC) at 0.33 equal initial relative proportion (PR : WC : RC = 8.2 : 2.3 : 7.5 kg/ha seed rate) maintained the botanical composition ratio over time, but WC and RC proportions fluctuated over time. The total legume proportion of the mixture was 0.38, 0.31 and 0.46 in the first, second and third years, respectively, was higher than the accepted level of 30% (Sanderson et al., 2012). The continuous persistence of sown species in a pasture sward indicates pasture persistence (Parsons et al., 2010; Tozer et al., 2011) for livestock production.

The characteristics of pasture swards were quantified in the ratio of sown botanical composition, showing that grass-legume functional components strengthened the pasture ecosystem for primary production and reduce weed infestation. A similar finding was recorded by Sanderson et al. (2004). PR was the most dominant and persistent species, ranging from 0.35 to 0.70 in binary and ternary mixtures along with perennial legumes. The proportion of PR in both binary and ternary mixtures, particularly those including RC and WC, showed fluctuations with a consistent trend of increase or decrease. This suggests an equilibrium in the proportion of PR when balanced with either RC or WC. WC and RC generally showed different growth characteristics in the mixture combination. WC is a slow-establishing species and is temporarily more persistent than RC (Black et al., 2017; Stewart et al., 2014). The presence of legumes improves the ecosystem's function (Finn et al., 2013b; Suter et al., 2015). The result showed that the PR*WC*RC in equal proportions in the mixture maintained the grass and clover proportion ranging from 0.50-0.64 and 0.31-0.46, respectively during the experimental period. In the typical pasture mixture, the PR*WC system in NZ maintains 10 to 20% white clover (Chapman et al., 1995b). The key factor is the maintenance of species proportion, which is important for the diversity effect (Nyfeler et al., 2009b) over time. PR in the optimal mixture of PR*WC*RC and PR*RC maintained the herbage biomass yield associated with the suppression of the weed fraction. Grasses generally have a competitive advantage over legumes, dominate the system and maintain productivity by maintaining the grass and legume proportion (Haynes, 1980). PR*P*WC*RC was also one of the four species mixtures, giving a higher yield of 1.85 t/ha. Even though this mixture had a lower WC proportion (< 0.08), RC contributed a greater sward yield, accounting for 32 to 45% in the second and third years, respectively.

Botanical composition dynamics of binary mixtures varied depending on the functional species combination. Based on some pasture mixture experiments, it was suspected that the species (growth rate) and intercepted light energy were the main factors affecting the botanical compositional changes of the species mixed swards (Caldwell, 1987; Keating and Carberry, 1993). The above factors were added as regression prediction components to quantify the botanical compositional changes in binary mixtures (RGRD). The prediction regression analysis identified that grasses and plantain in the mixture community exerted a positive effect, and clover components negatively influenced the RGRD of binary mixtures. The magnitude of the effect differed between species, and grasses exerted greater influence than legumes. The intercepted light in the regression prediction identified that the intercepted light positively affected the botanical composition of binary mixtures. Competition for light interception in the multi-species canopy is a major factor responsible for the shifting of botanical composition (Keating and Carberry, 1993) and biomass production. The canopy structure of component species is an important factor in determining the competitive ability of the plants (Caldwell, 1987).

The botanical composition of species mixed pasture swards can be managed with a minimum degree of changes until pasture renovation (this experiment evaluated the first four years). The selection of species and their relative proportion in the mixture determines the botanical composition of herbage materials over time and sward productivity. The identified species mixed pasture swards (PR*WC*RC; PR*RC) for improved production over the years was from grasses and legume mixtures.

5.6 Conclusion

1. The biomass production potential of monocultures and species mixed swards differed in the first and second comparison periods (1st to 2nd and 2nd to 3rd years), Grasses dominated and produced higher biomass over the comparison period. Plantain and legume species showed a relative abundance reduction over both comparison periods. The intra-mixture RGR comparison for biomass production showed that the potential for biomass production was reduced in the 3rd year compared with the second year, accounting for 75% of the mixture communities.

2. The sown proportion of mixture communities increased with increasing species richness. The grass: legume proportion of PR*WC, PR*RC and PR*WC*RC were 0.73:0.20, 0.55:0.33 and 0.54:0.38, respectively. The identified mixture combinations, such as PR*WC, PR*RC and PR*WC*RC, maintained the balanced of species for over three years.

3. RGRD value comparisons between binary mixtures in the first and second comparison periods showed positive and negative values depending on the growth potential of component species. PR*WC and PR*RC showed a different degree of botanical composition fluctuation, mainly from the associated clover component. Grass and clover composition the binary and ternary mixtures showed the oscillating dynamics over three years.

4. The component species proportions in binary and ternary mixtures maintained the sown species proportion from PR, WC, and RC. Identified mixtures were PR: WC, PR*RC, and PR*WC*RC. These mixture communities maintained the grass to legume proportion of 0.53-0.55 and 0.33-0.38 over three years, respectively, mainly the WC component fluctuated.

5. RGRD modelling and ternary contour plots identified that an equal proportion of PR, WC and RC species maintained the component proportion over three years.

6. The compositional changes in binary mixtures were driven by the RGR of the functional component species and intercepted light energy. The strongest influence was from species rather than the intercepted light energy.

Chapter 6

Light interception and Radiation Use Efficiency of species mixed pasture swards

6.1 Introduction

Changes in species mixed pasture sward dry matter yields and botanical composition were discussed in Chapters 4 and 5. This chapter aims to determine whether those responses were closely associated with the primary process of “canopy light interception”. Differences in canopy light interception are the major physiological factor that governs productivity and are considered in pasture growth analysis (Biscoe and Gallagher, 1977). This chapter describes light interception and light (radiation) use efficiency (RUE) in these formulated pasture sward communities. Light interception of monocultures and multi-species swards are compared with the binary and centroid mix, as well as RUE and its relationship with temperature. The null hypothesis is that there is no difference in light capture, and the efficiency of its use between monocultures and species mixtures. The alternative is that the efficiency of light interception, differs due to differences in canopy formation, and this explain the yield differences observed in Chapter 4.

The selection of mixture component species and their relative abundance in the seed mixture formulation were outlined in Chapter 3. This is the basis for canopy formation to achieve efficient light interception and improve pasture production. The expectation is that a positive diversity effect on canopy formation leads to efficient light interception. The photosynthetically active tissues, mainly green leaves, play a crucial role in light interception, driving crop/pasture growth and yield (Monteith et al., 1977). Thus, the mixture combination of species, and optimal proportion of component species in the seed mixtures that improved pasture responses reported in Chapter 4 are expected to be directly proportional to the canopy intercepted amount of light and photo-assimilates allocated to the different plant parts (Cooper, 1970; Moot et al., 2021a). In the multispecies pasture sward, light interception depends on canopy cover, determined by the inclusion of competitive species in the mixture (Fessel et al., 2016) and how efficiently light is used for biomass production (Monteith, 1972). Connolly et al. (2009) suggested that an increasing number of species in natural grassland improved biomass productivity. The amount of light intercepted by the sward is determined by the developmental stage of the canopy, Leaf Area Index (LAI) and canopy architecture. This implies that enhanced light capture of multi-species swards would enable an increased stocking rate on a farm and lead to higher profitability. Further, pasture research suggests that grazing should be initiated when forage cover captures 80 to 90% of incident radiation

(Sollenberger, 2018). Canopy fPARi is an important canopy characteristic to evaluate the light interception and biomass production potential of swards. Therefore, determining the pre-grazing fraction of light interception is part of this chapter.

Once the light is intercepted, the amount of biomass produced depends on RUE. Within the plant communities, intraspecific and interspecific variations exist in biomass production efficiency or photosynthetic efficiency (Green, 1987). For instance, grasses are expected to have higher RUE than legumes (Faurie et al., 1996) due to the relatively higher cost for legumes of producing more proteins. In multi-species pasture production systems, mixing different species or pasture species alters both radiation interception and RUE of swards (Elhakeem et al., 2021). The improvement of RUE may be from N fixation from symbiotic association, and utilization of fixed N by non-legume component species in the mixture (Nyfeler et al., 2011). The expectation is that the canopy of species mixed swards intercepts more light in addition to added N to the system improve pasture production. In this irrigated environment, the main abiotic factors that influence pasture production are light interception and temperature, which affect the canopy photosynthesis rate (Monteith, 1972). This experiment evaluates the sward canopy potential for light interception and determine how RUE is affected by temperature.

Analysing the competition for light in a diverse species of mixed pasture communities and its effect on DM production is a long-term need in pasture research. This chapter aims to explain the "physiological mechanism underlying the improved sward responses in Chapter 4" based on light interception and RUE.

Theis related to objectives (9) to quantify the sward canopy fraction light (PAR) interception, (10) to quantify and compare the RUE of swards including functional species, (11) to quantify the temperature effect on RUE of selected pasture swards, and (12) to quantify the identity and diversity contribution of species mixed canopy and RUE of swards.

6.2 Materials and Methods

The data for this chapter were collected from six monocultures and all 15 binary mixtures plus the centroid mixture. This is because these treatments produced the greatest differences in yield (Chapter 4).

The methods used to quantify the fraction of light interception (PARi) and intercepted light energy were described in Sections 3.5.4. For this purpose, the Sunscan canopy analyser (Type SS1, Delta-T devices Ltd, UK) was used to measure the fraction of light interception. The first Sunscan reading was taken approximately 3 to 7 days after the commencement of each new regrowth cycle. The aim was for a minimum canopy height of 2.5 cm, which is required to cover the Sunscan sensor height of 1.5

cm. At the beginning of each growth cycle, the fraction of light interception value was calculated based on the NDVI value of canopy cover proportionally interpolated to calibrate the Sunscan readings (Talamini, 2022). The value of fPARi for each day for each plot was obtained by interpolating between two consecutive Sunscan readings. The sward recovery and canopy potential for light (PAR) interception were assessed by considering the canopy fraction PAR interception (fPARi) at the mid-point of each re-growth cycle and the fraction of PAR interception measured on or within a day of grazing (pre-grazing). Canopy light (PAR) accumulation did not include the first grazing followed by the 2020 winter (28/07/2020). The relevant total biomass accumulation included seven regrowth/grazing cycles (14/09/2020, 21/10/202, 30/11/2020, 12/01/2021, 17/02/2021, 25/03/2021 and 25/05/2021). To estimate the incoming incident photosynthetically active radiation (PAR), the total daily incident radiation obtained from Broadfield meteorological station (2 km north of experimental site) was multiplied by a factor of 0.5 (Coops et al., 1998; Meek et al., 1984).

6.2.0 Statistical analysis

Regression analysis was performed on a plot basis for the 2020/21 season to determine the relationship between herbage biomass and light interception for each harvest and each species. Because the measurements for all harvests were made for each plot, this is a 'repeated measures' data set, with Harvest as the 'repeated' factor. For 'repeated' factors, it is likely that measurements from the same plot will be more related to each other than measurements from different plots, and that measurements at harvests closer together in time will be more highly correlated than measurements further apart. Since the standard simple statistical analysis approaches (example-ANOVA) assume the same variance plus the same correlations across all treatment combinations, adjustments need to be made that allow for the non-constant variance and correlations. If the adjustments are not made, standard errors will generally be spuriously small, and p-values similarly.

The simplest approach is to treat the 'Repeated' factor as a 'split plot' (or nested) treatment. This method needs further adjustment because the split plot method assumes constant correlations. The most straight forward approach is to fit various other correlation structures and choose the 'best' fitting (that with the smallest REML deviance). Correlation structures cannot be estimated with ANOVA. Instead, the various models can be fitted with REML (Restricted Maximum Likelihood (REML, Payne et al., 2017). The structure with the smallest REML deviance where there was no significant reduction (by a X^2 test) in comparison with a simpler model was chosen. That structure was the 'Unstructured' model, which has a different variance estimated for each Harvest, and different co-variance between each pair of harvests.

Once an appropriate correlation structure had been selected, further analyses were carried out as for RUE, except that harvest and interactions between species or contrasts were included as fixed effects, with all interactions between harvest, species or contrasts included. ‘Rep’ and ‘Plot’ were included as random effects: these were found to be important when assessed using a X^2 test of the REML deviance on dropping a term. Fixed effects were assessed with F-tests of Wald statistics, using the Kenward & Roger estimate of the denominator degrees of freedom (Li and Redden, 2015).

Two variables were analysed, the accumulated intercepted PAR (Cu PARi) which gave the mean for each harvest, and then Cu PARi/re-growth days to give Cu PARi per regrowth day. For simplicity, the same Random and Correlation model was used for both.

For all analyses, means and associated 95% confidence limits were obtained from the results of the analyses.

For RUE the data were analysed with analysis of variance (ANOVA), with Replicates included as a blocking structure. Initially, Species were included as a treatment factor. In a second ANOVA, contrasts between the Species were included as follows:

Type/(Mono+Binary Centroid/Binary)

Where the factors compare:

Type: the mean of monocultures with the mean of all mixtures

Monoculture: amongst the six monocultures

Binary Centroid: the mean of the binary mixtures with the centroid mix

Binary: amongst the 15 binary mixtures

In a third analysis, each binary mixture was compared with the two monocultures comprising that pair (example-. pair RC-SC with monocultures RC and SC). A new analysis was performed for each pair, with treatment formula:

Pair Orthogonal comparison /(BinMon/Mon-aMon-b+Other)

Where the factors compare:

Pair Orthogonal comparison: the mean of chosen pair and constituent monocultures with all the other mixes

Binary Monoculture: the mean of the binary pair with the mean over the two constituent monocultures

Mon-a Mon-b: one of the monocultures with the other monoculture (Example- for pair RC-SC between RC and SC)

Other: amongst all other mixes. This is needed to fit the same full model as is fitted with the simple Species model done first.

The means and associated standard errors were saved from the first (Species) analysis. 95% confidence limits were then calculated for each mean. Note that the confidence limits for two means can overlap by as much as half and the means still be significantly different (Frampton pers. comm. 2023).

These analyses were all carried out with Genstat (Payne et al., 2020).

The RUE response to temperature was also assessed using the mean temperature of the regrowth periods, obtained from Broadfield meteorological station (2 km north of experimental site). Data were analysed with Analysis of Variance (ANOVA), and replicates were included as blocking structures. Finally, DI model analysis quantified the identity, and diversity effect (mixture effect) on canopy fraction for PAR interception and RUE as proposed by Kirwan et al. (2009).

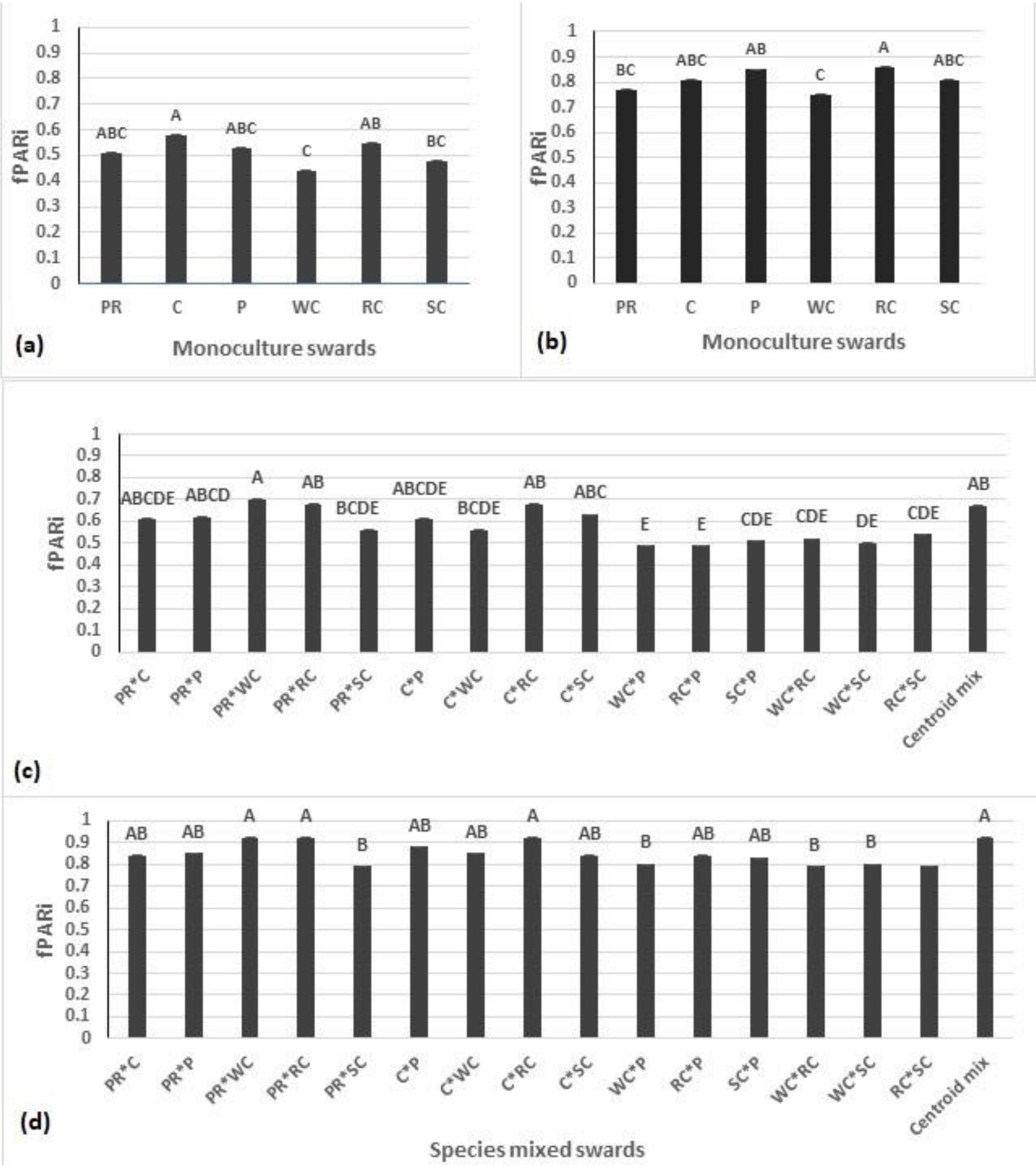
6.3 Results

6.3.1 **Canopy fraction for light interception (fPAR)**

Figures 6.1 a and c show the fPARi in the middle of the re-growth cycle for monocultures and species mixed swards. Similarly, Figures 6.1 b and d show the fPARi of the swards immediately before grazing (pre-grazing). The pre-grazing fPARi differed (p=0.05) among monocultures, species mixed swards, and re-growth cycles. Among the monocultures, fPARi at the mid of regrowth cycle was higher for cocksfoot than WC and SC (Figure 6.1 a). By the end of the regrowth cycle RC had higher fPARi than WC and PR. At the mid regrowth cycle, the canopy fraction of light interception value of monoculture swards and species mixed pasture swards were, on average 0.51 and 0.58 (Figure 6.1 a and c), respectively. The pre-grazing values were were 0.80 and 0.84, respectively (Figure 6.1 b and d). The pre-grazing canopy fraction interception values for WC, PR, SC, C, P and RC monocultures were 0.75, 0.76, 0.81, 0.81, 0.85 and 0.86, respectively. For the binary mixtures, the fPARi at the mid growth for the PR*WC, PR*RC, C*RC and the centroid mix were higher than any of the binary mixes that did not contain a grass (Figure 6.1 c). By the end of the rotation, the fPARi at pre-grazing was higher (P<0.05) for PR*WC, PR*RC, C*RC, and the centroid mix than PR*SC, WC*RC, WC*SC, WC*P, and RC* SC (Figure 6.1 d).

The fPARi value differed with the regrowth cycles (P<0.01) but was not different among monocultures and binary mixtures (Figure 6.2) over the regrowth cycles. Regardless of sward type the fPARi was more than 0.85 in late spring (30/11/2020), mid-summer (12/01/2021) and late autumn (25/05/2021). The lowest value was at the beginning of autumn (25/03/2021).

Figure 6.1 Canopy fraction of PAR interception for monoculture and species mixed swards at the middle of regrowth cycles (a & c) and at the pre-grazing (b &d) in a diverse species mixed pasture experiment at Lincoln University, Canterbury. Means that do not share the same letters are significantly different at P=0.05



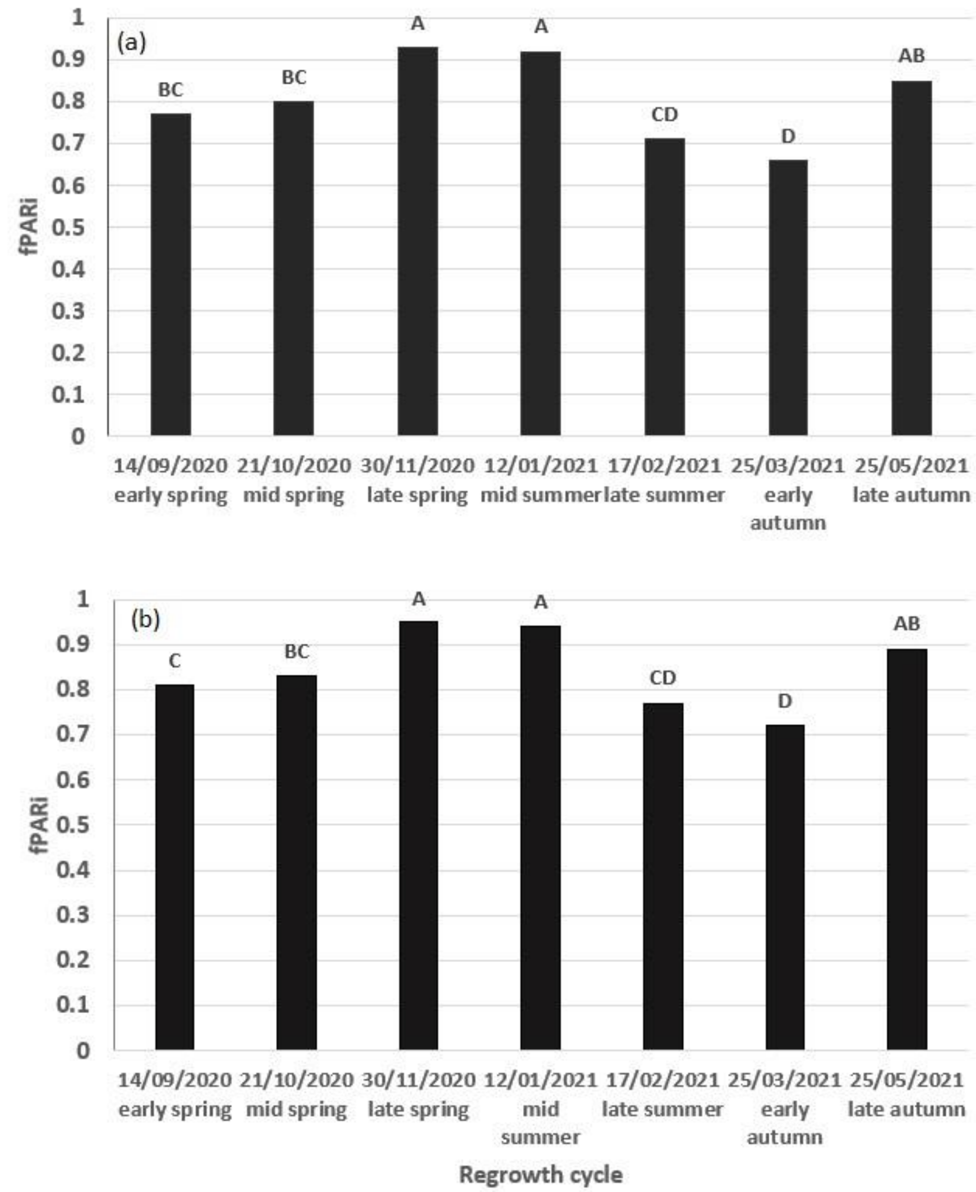


Figure 6.2 Pre-grazing fPARi value of monoculture swards (a), and species mixed swards (b) over the re-growth cycles in a pasture diversity experiment (2020/21) at Lincoln University, Canterbury, New Zealand. Means that do not share the same letter are significantly different.

6.3.2 Mixture contribution on canopy PARi and intercepted energy of swards

Figure 6.3 shows the amount of intercepted PAR of each of the binary mixtures in relation to their monoculture performance over the regrowth cycles. The main cause of the differences was due to the fPARi of the swards canopy. On average, the binary mixtures, intercepted more PAR (119 MJ M^{-2}) than the average of monocultures, at 102 MJ M^{-2} ($p<0.05$). The binary mixtures intercepted above or below average of the intercepted PAR depending on the associated functional species of mixture and time of the year as shown by the regrowth cycles (Figure 6.3). Average intercepted PAR energy per regrowth cycle (2020/21) of the centroid mixture did not differ (P<0.01) from the binary mixtures. The differences in intercepted PAR energy were due to the differences in canopy formation of species mixed swards.

The average pre-grazing fPARi over the re-growth cycles was subjected to DI model. The quadratic (p=0.01) model analysis revealed that the component species proportion in binary mixtures influenced canopy fPARi, and the mixture contribution on fPARi was either positive or negative, depending on the functional species of mixtures. The positive interaction coefficient value (p=0.05) of PR*P, C*RC, PR*WC, and PR*RC indicated synergistic interaction in canopy formation, which improved the fPARi (Appendix P). The model analysis estimated an additional interaction canopy contribution of fPARi value of 0.10, and 0.47 for PR*RC and PR*WC mixtures, respectively. Figure 6.3 generated from GENSTAT clearly shows the species contribution on intercepted PAR energy of binary mixtures overtime. A positive mixture effect was observed for PR*P, PR*WC and PR*RC and was prominent in the latter part of the 2020/2021 production year, mainly in regrowth cycles 6, 7 and 8 (first regrowth cycle is not shown in Figure 6.3). Negative values were mainly found in the mixture communities that included SC and P as component species. The results over time showed that the monoculture of WC intercepted the least Cu PAR in the 5th and 6th rotations.

Among the best mixture communities, PR*P and C*RC, intercepted on average, more PAR than the PR*RC and PR*WC, which intercepted 124-128 MJ M^{-2}. Further, the mixture contribution (diversity contribution) on canopy intercepted PAR energy for C*RC, PR*RC, PR*P, and PR*WC were 22, 31, 40, and 59% higher than the proportional contribution of monoculture component species in the mixtures. The reduced WC performance in late summer and autumn reduced the intercepted PAR energy in WC*RC, WC*SC and WC*P binary mixtures.

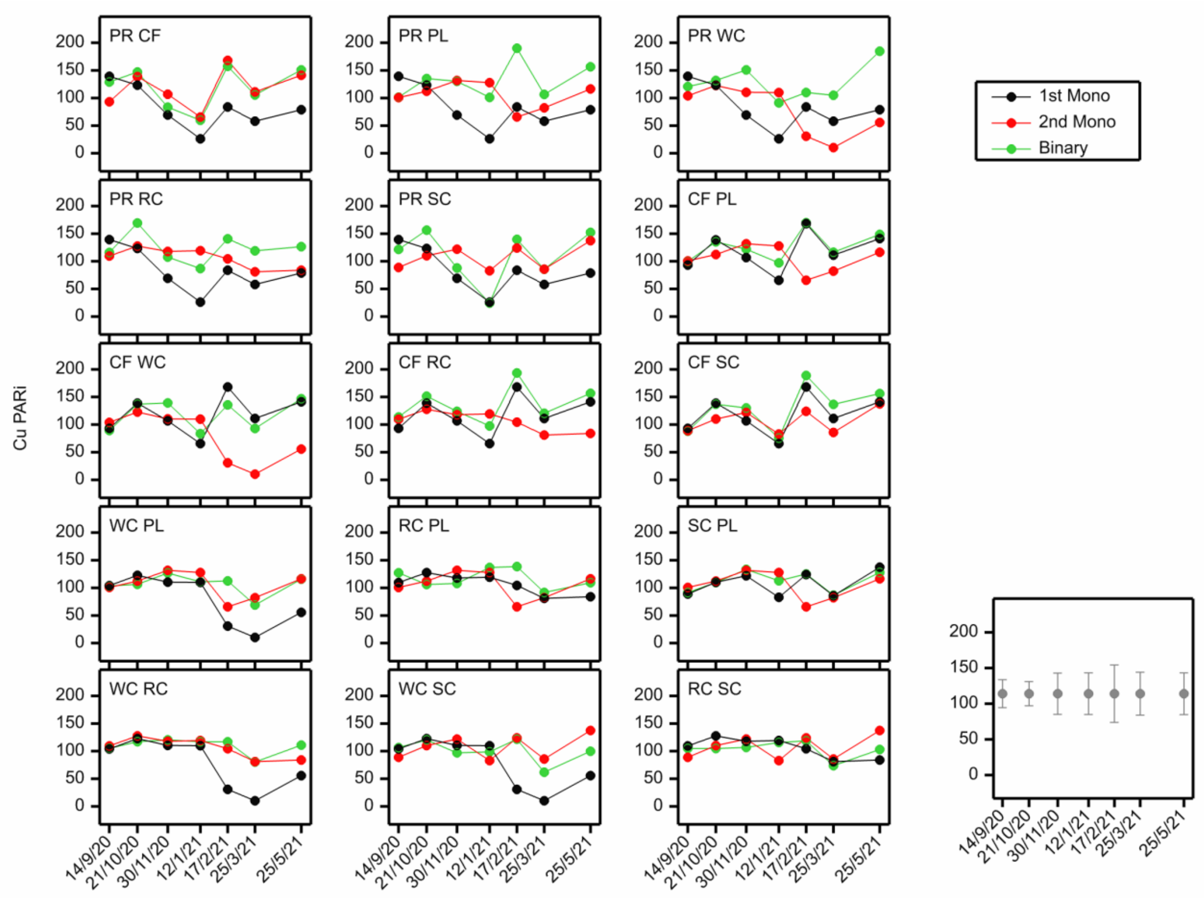


Figure 6.3 Mean intercepted PARi energy of binary mixture (green) and their component species (black and red) in a pasture diversity experiment at Lincoln University, Canterbury, New Zealand. Error bars are 95% confidence limit.

6.3.3 Radiation Use Efficiency (RUE) of swards and mixture contribution

The RUE of the functional group species differed ($p < 0.05$) among them. There was a strong (>99%) linear relationship between cumulative biomass (g) and cumulative PARi (MJ M^{-2}) for all functional species. RUE of perennial grasses, herbs and perennial clovers were 1.25, 1.67 and 1.69 g DM MJ^{-1} (Appendix N). This shows the perennial clovers produced 16% more ($P<0.05$) biomass per unit intercepted PAR energy than the grasses. These functional species were used to formulate diverse species mixed pasture seeds for improved herbage biomass production. For example, the grass-grass and grass-herb association had a lower RUE value (1.25 g DM MJ^{-1}) than the grass-clover association, of 1.50 g DM MJ^{-1}.

The RUE value across the pasture swards is presented in Figure 6.4. RUE calculations excluded the first grazing cycle (28/07/2020), followed by winter. Biomass yield showed a positive linear relationship with intercepted PAR for all swards. There were differences ($P<0.05$) among monocultures but overall, they differed ($P=0.01$) from the binary mixtures. The RUE of monocultures ranged from 1.18 g DM MJ^{-1} for cocksfoot to 1.76 g DM MJ^{-1} for sub clover. The RUE of PR, P, RC, and WC were intermediate at 1.31, 1.54, 1.56 and 1.61 g DM MJ^{-1}, respectively (Figure 6.4).

The RUE of binary mixtures differed (P<0.01) among them, and ranged from1.02 g DM MJ^{-1} in C*SC to 1.79 g DM MJ^{-1} in RC*SC, respectively (Figure 6.5). The RUE of the binary mixtures was not different from the centroid mixture. The mean RUE value for some mixes was higher than the monocultures and some were lower than the average of component species. The most efficient canopy formation for PAR interception and biomass production binary mixture combinations were PR*WC, PR*RC, and C*RC. The binary mixture communities which had lower RUE than the monocultures were in C*SC and SC*P (P<0.01). These lower values suggest a negative interaction between the component species or reduction of relative abundance. In contrast, The RUE values for PR*WC and PR*RC were 1.55 and 1.68 g DM MJ^{-1}, and higher (P<0.01) than the average of monocultures. In most cases the binary combination improved the RUE of swards based on the functional component species. Among the binary mixtures, higher RUE values were observed in PR*WC, PR*RC, C*WC, RC*P and RC*SC. The mean RUE value of the above mixtures was 1.58 g DM MJ^{-1}, which was higher than the average of the component species (1.46 g DM MJ^{-1}).

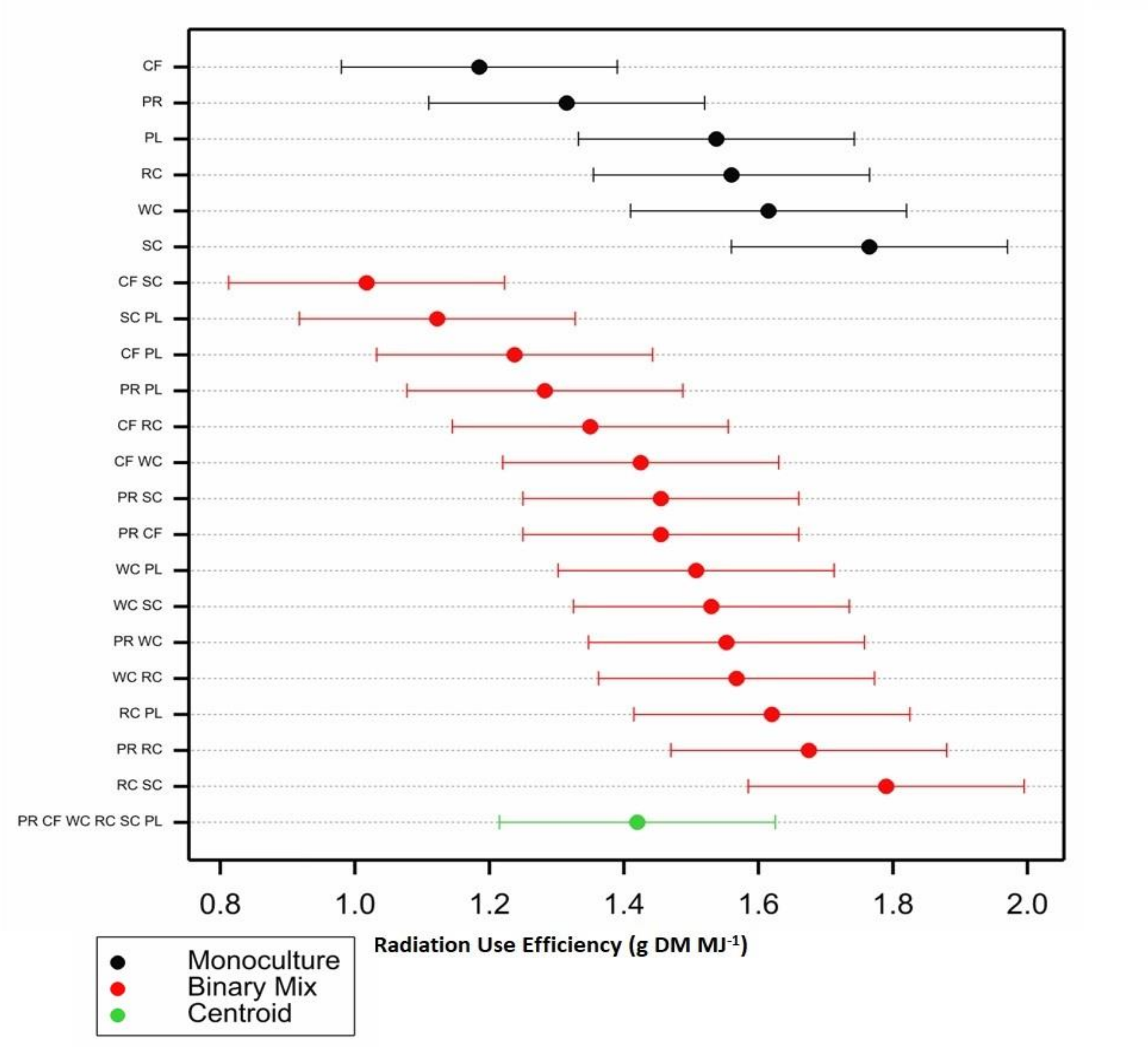


Figure 6.4 Mean Radiation use efficiency of pasture swards in a diverse pasture species mixed experiment under irrigated and sheep grazing conditions (2020/2021) at Lincoln University, Canterbury, New Zealand. Error bars are 95% confidence limits.

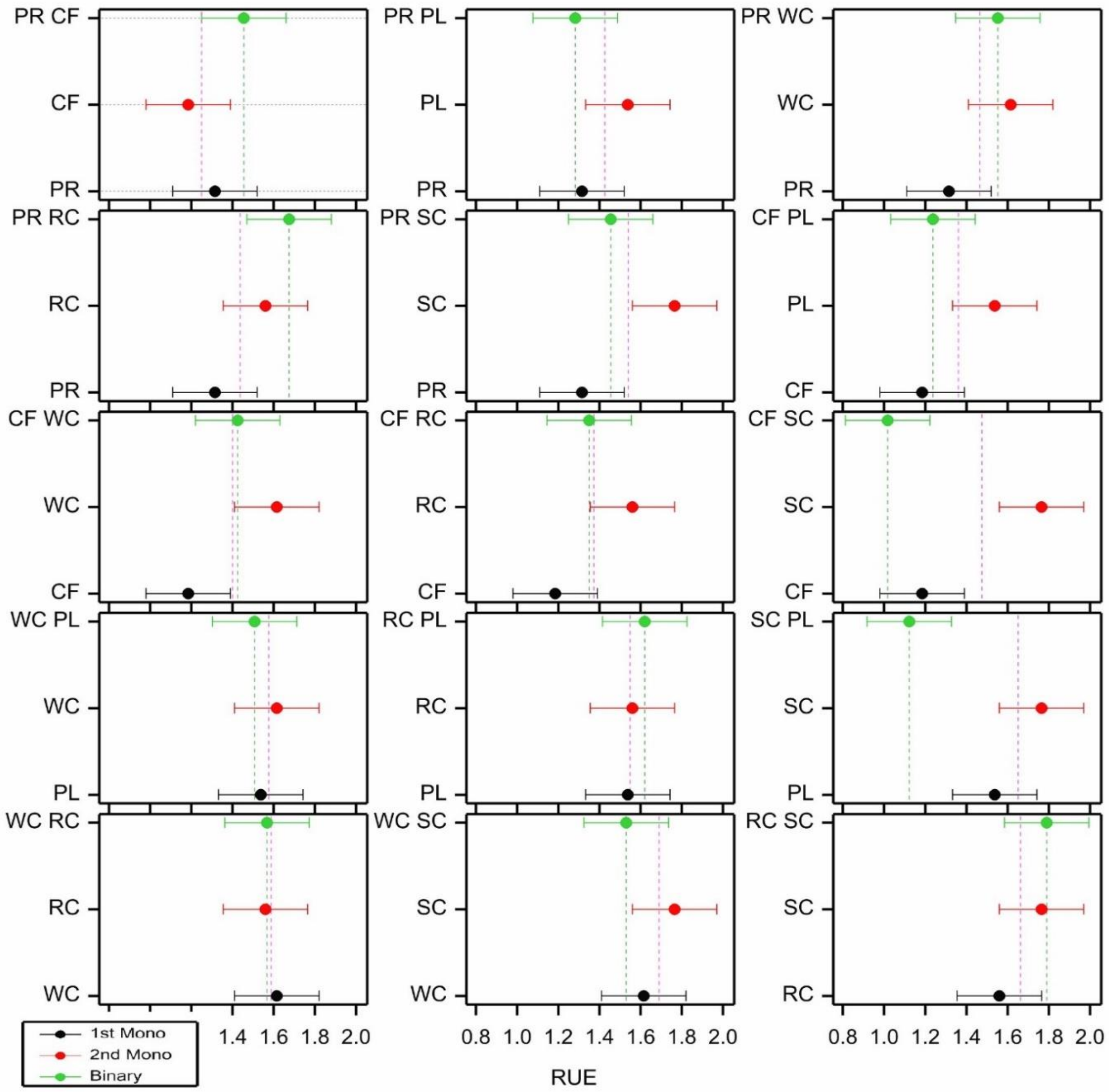


Figure 6.5 Comparison between mean RUE value of binary mixtures (red) and their mixture component species (red and black) with droplines indicating RUE value in a diverse pasture species mixed experiment at Lincoln University, Canterbury, New Zealand. Error bars are at 95% confident limit.

The effect of species proportion on RUE efficiency of mixture communities and mixture contributions is quantified in Appendix Q. The component species proportion of mixtures influenced (P=0.01) the RUE value of swards. The significant linear model indicated that the monoculture species differed (P<0.01) among them. The model coefficient values for tested mixtures were positive and negative depending on the component species in the mixture. Both repeated measure ANOVA analyses output for re-growth cycles and DI model analyses for RUE resulted in positive and negative contribution of mixtures (Appendix Q) as shown in Figure 6.5. The interaction contribution for PR*WC and PR*RC provided the additional estimated RUE value of 0.05 and 0.24 g DM MJ^{-1}, though the interaction between binary component species was not significant.

6.3.4 **Factors that influence RUE.**

The biotic and abiotic factors can influence photosynthesis and therefore RUE and DM production. The main factors considered in this experiment were herbage N content, weed content, volunteer white clover (VWC%), soil moisture and temperature. The herbage N content in the 2020/21 production year ranged from 2.6% in PR to 3.9% in RC. The mean herbage N content of PR*WC and PR*RC in 2020/21 were 3.3 and 3.6%. The N content of the herbage materials of selected swards was positively correlated (r=0.48) with RUE. The herbage DM yield, weeds, and VWC fraction, for the 2020/21 season are presented in (Appendix O). The RUE of perennial legumes monoculture swards such as RC and WC were 1.56 and 1.62 g DM MJ^{-1}, even though the above ground green biomass had 22% and 47% weeds, respectively. The weed content of the plantain monoculture was 37%, and the mean N content was 3.7%, with a RUE of 1.54 g DM MJ^{-1}. The SC monoculture had 94% weeds, of which 16% was VWC. The N content of the SC monoculture herbage was 3.1%, with an RUE of 1.76 g DM MJ^{-1}. The centroid mixture produced an average of 1.9 t/ha (2020/21) herbage biomass yield, including 3% unsown species, and it had an RUE of 1.42 g DM MJ^{-1}. The RUE values of PR*WC and PR*RC were 1.55 and 1.67 g DM MJ^{-1}, respectively. The RUE values of these mixtures were higher than grass monocultures. The weed component in herbage green biomass intercepted light and contributed to biomass yield. The commonly identified weed species were *Poa annua* L., chickweed (*Stellaria media* L.), fathen (*Chenopodium album* L.), Wireweed (*polygonum aviculare* L.) Cleavers (*Galium aparine* L.) and willowweed (*persicaria maculosa* L.). The perennial weeds were dock (*Rumex obtusifolius* L.), and dandelion (*Tarraxacum officinale* L.).

Table 6.1 RUE of pasture swards, yield/re-growth cycle (t/ha), Weeds, Volunteer White Clover (t/ha) and herbage N content (%) in a multi-species mixture experiment in 2020/21 production year at Lincoln University, Canterbury, New Zealand.

Sward type	RUE (g DM MJ^{-1})	Biomass yield (t/ha)	Weeds (t/ha)	VWC (t/ha)	Herbage N content (%) 2020/21
PR	1.32 ± 0.03	1.20 ± 0.13	0.01 ± 0.00	0.00 ± 0.00	2.6 ± 0.14
C	1.19 ± 0.03	0.98 ± 0.09	0.02 ± 0.01	0.02 ± 0.01	2.7 ± 0.03
P	1.54 ± 0.06	1.39 ± 0.12	0.79 ± 0.06	0.36 ± 0.09	3.7 ± 0.16
WC	1.62 ± 0.02	1.37 ± 0.11	0.94 ± 0.06	0.00 ± 0.00	3.6 ± 0.17
RC	1.56 ± 0.04	1.59 ± 0.16	0.54 ± 0.16	0.24 ± 0.04	3.9 ± 0.07
SC	1.76 ± 0.14	1.50 ± 0.14	1.26 ± 0.14	0.15 ± 0.03	3.1 ± 0.11
PR*WC	1.55 ± 0.06	1.89 ± 0.34	0.09 ± 0.02	0.00 ± 0.00	3.3 ± 0.10
PR*RC	1.68 ± 0.09	1.98 ± 0.19	0.08 ± 0.02	0.16 ± 0.03	3.6 ± 0.06
Centroid Mix.	1.42 ± 0.06	1.88 ± 0.08	0.06 ± 0.02	0.00 ± 0.00	3.6 ± 0.02

Annual rainfall during the experiment (2017/2018, 2018/1019, 2019/2020, 2020/2021) was 745, 514, 475 and 519 mm, respectively. Irrigation and rainfall replenished soil moisture. In the 2019/2020 and 2020/2021 seasons, above average rainfall returned the soil to field capacity on (01/06/2019, 29/06/2020, 8/11/2020 and 30-31/05/2021). The water holding capacity of the experimental site is 300 mm (Black and Lucas, 2018). Daily evapotranspiration, irrigation and rainfall of the experimental site is given in Figure 6.6. Irrigation water application rate was 15 mm/hour and the total amount applied during irrigation is presented in Figure 3.1. Table 6.2 shows the average soil moisture content across each re-growth cycle. Soil moisture deficit for monoculture species showed that the water requirement changed throughout the growing season as a cyclic pattern related to the seasonal weather pattern. PR, RC, P and WC monocultures reached a soil moisture deficit level of more than 200 mm/m compared to SC and C (Figure 6.7). The average soil moisture profile of herbs is given in Appendix T.

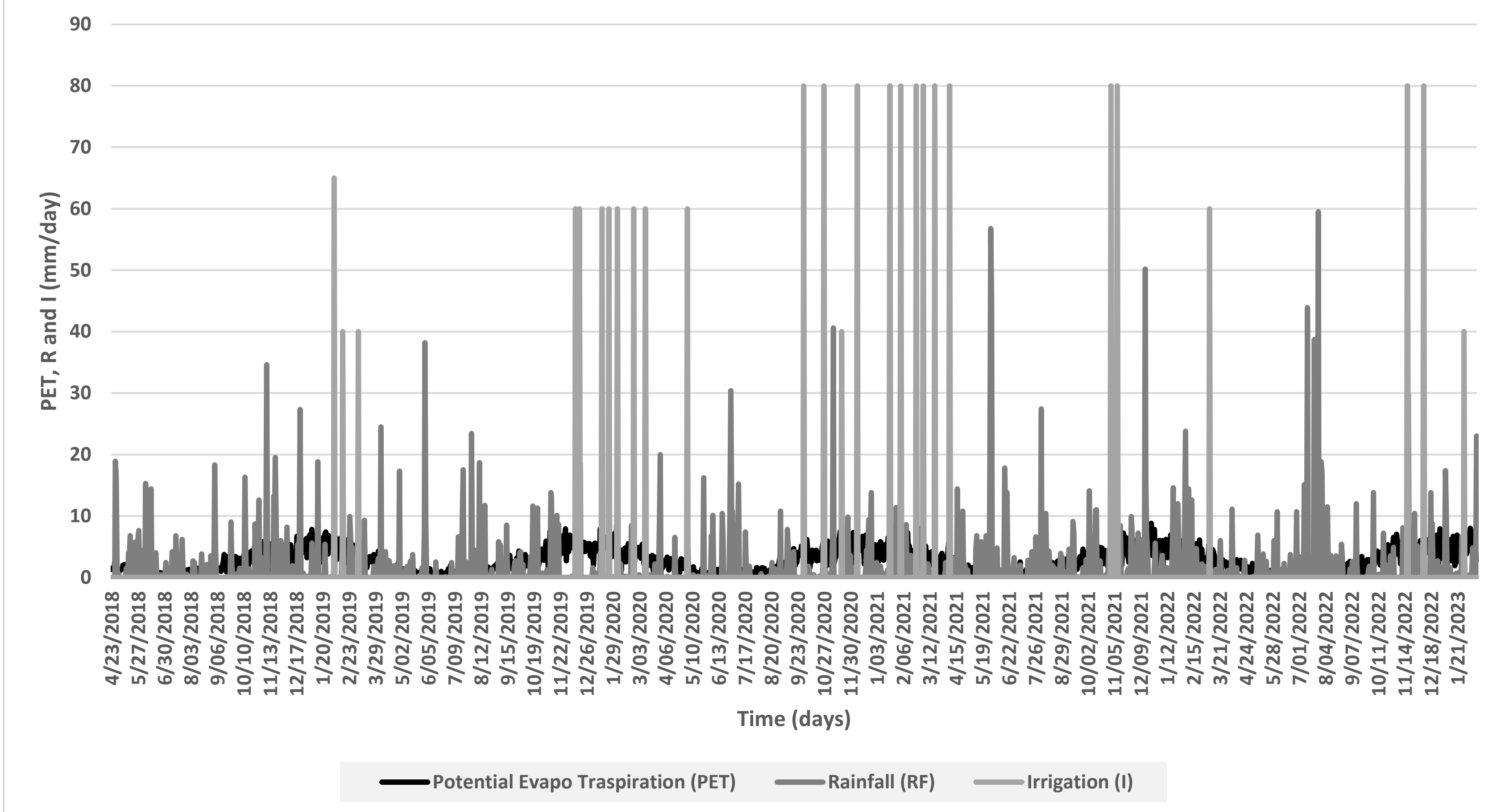


Figure 6.6 Irrigation (mm), Rainfall (mm) and Potential Evapotranspiration of I8 experimental site at Lincoln University, Canterbury, New Zealand (2018 to 2023).

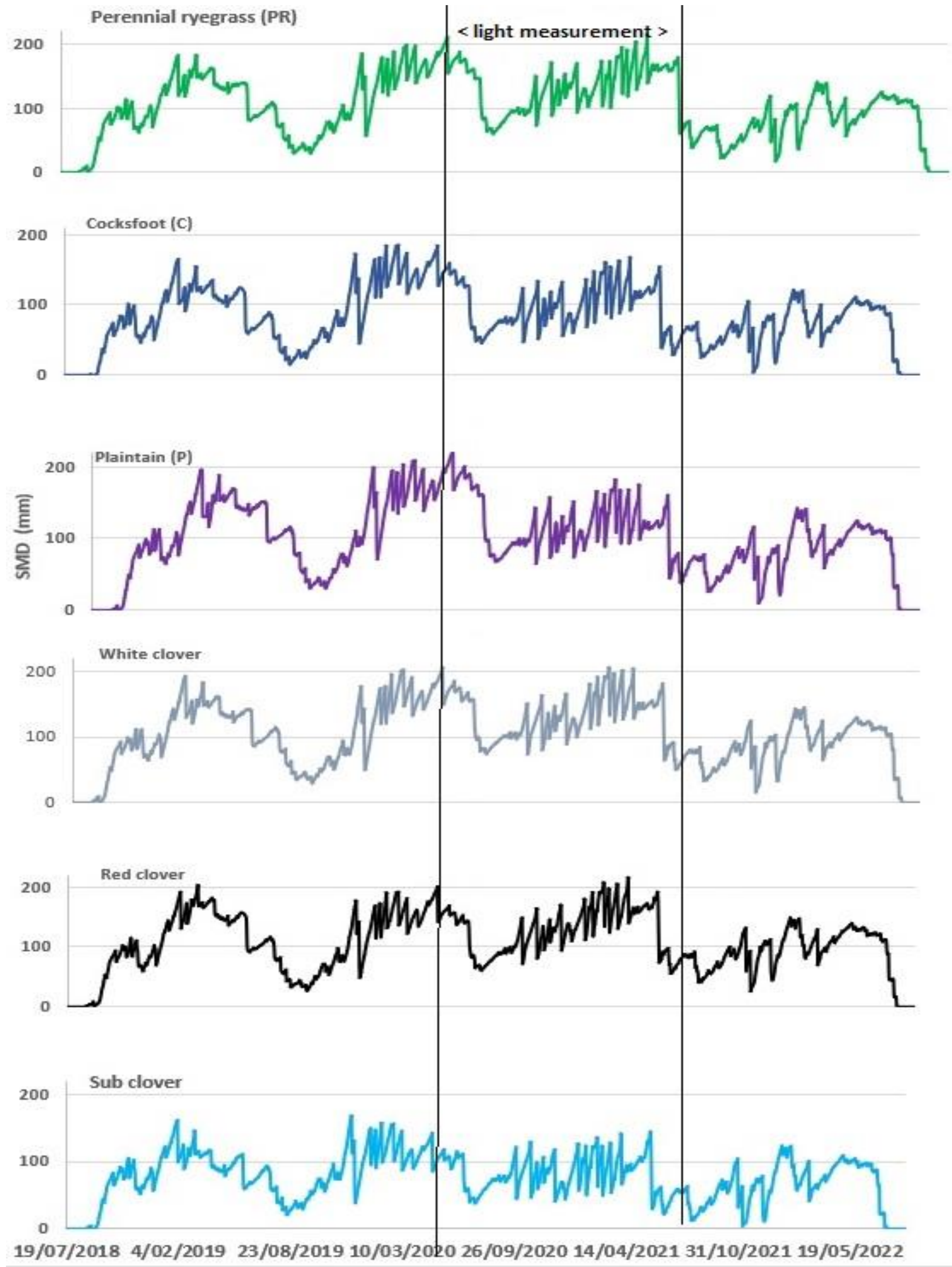


Figure 6.7 Water deficit calculated for monoculture species in a pasture diversity experiment at Lincoln University, Canterbury, New Zealand (2018-2023).

Table 6.2 Soil water balance in re-growth cycles in 2020/21 growth year in a diverse species mixed pasture experiment at Lincoln University, Canterbury, New Zealand

Sampling date	Regrowth Cycle	Average T (°C)	Cumulative ET (mm)	Irrigation (mm)	Rainfall (mm)	Soil moisture deficit (mm)
14/09/2020	17	8.6	45.8	0	31	0
21/10/2020	18	11.9	116.6	80	4.4	0
30/11/2020	19	13.7	158.7	40	69.4	49.4
12/01/2021	20	15.5	177.7	80	56.4	41.4
17/02/2021	21	16.5	210.3	160	37.4	0
25/03/2021	22	16.1	190.2	240	7.6	0
25/05/2021	23	12	142.7	80	62.6	0.17

Plate 6.1 Weed infestation in diverse species mixed pasture experiment at Lincoln University, Canterbury, New Zealand. (A) Weeds in experimental plot (B) Annual weeds in red clover monoculture.

6.3.5 **Effect of Temperature on Radiation Use Efficiency of swards**

The mean temperature across the regrowth cycles in 2020/2021 season ranged from 8.6 ˚C to 16.5˚C, with a minimum and maximum temperature range of 3.6 ˚C (17/08/2020) to 24.8˚C (24/02/2021). The RUE and T relationship for monoculture species did not differ ($p < 0.05$) among the monocultures. However, temperature did show a positive ($P < 0.01$) effect on RUE of most species with coefficients of determination between 0.38 and 0.59. Photosynthetic responses (RUE) to unit temperature ranges from 0.04 to 0.05 g DM MJ $°C^{-1}$ for grasses, from 0.05 to 0.07 g DM MJ^{-1} $°C^{-1}$ for perennial legumes (Appendix R).

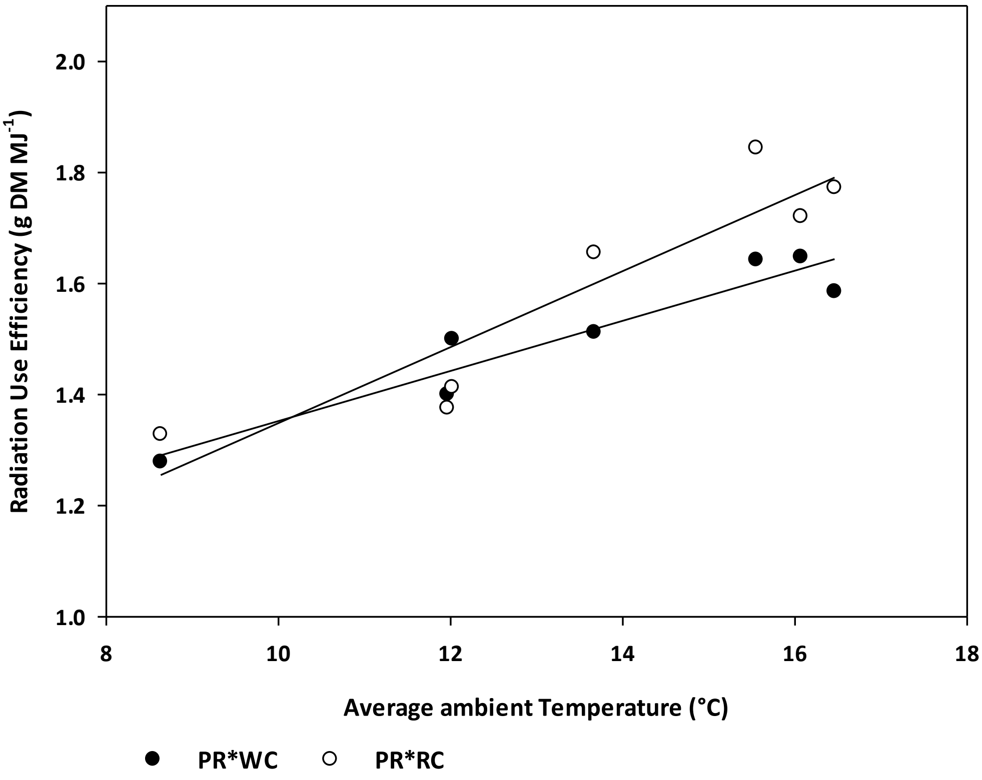


Figure 6.8 RUE as a function of temperature for PR*WC and PR*RC mixtures in 2020/21 growth season at Lincoln University, Canterbury, New Zealand.

The sensitivity of RUE to temperature was not different for PR and RC, value was 0.05 g DM MJ^{-1} $°C^{-1}$. RUE of PR*WC and PR*RC were regressed against the average ambient temperature in each regrowth cycle and showed a positive ($p < 0.01$) effect on RUE. The relationship between RUE and temperature was moderate (R^2 > 34-46%) for these mixtures. The values were 0.05 g DM $MJ^{-1}°C^{-1}$ for PR*WC, and 0.08 g DM $MJ^{-1}°C^{-1}$ for PR*RC (Figure 6.8). The RUE and temperature relationship for PR*WC binary mixture showed less sensitivity to temperature than the PR*RC binary mixtures (Appendix S).

6.4 Discussion

This chapter outlines how species mixed pasture swards enhanced the light interception and radiation use efficiency, to explain the increased biomass of mixtures over monocultures observed in Chapter 4. These results are consistent with previous diversity experiments that show enhanced community-level light interception (Sapijanskas et al., 2014; Zhu et al., 2015), and consequently herbage biomass production (Keating and Carberry, 1993). On average, species mixed pasture swards intercepted 7% more light and produced 16% higher DM yield (2020/21) than the monocultures. The RUE of binary mixtures such as PR*C, PR*WC, PR*RC, C*WC, RC*P and RC*SC was higher than the mean of component species (1.25 to 1.65 g MJ^{-1}) and differed depending on the component species in the mixture. Interspecific variation existed in the RUE value of monocultures. This experiment confirms that biomass was improved by greater light interception from mixing the species, and particularly by blending legume and non-legume component species. The herbage biomass productivity is the product of the canopy intercepted PAR energy, and the light use efficiency (Jamieson et al., 1998; Keating et al., 2003). Herbage biomass production differed between the mixture communities, but the most successful binary mixture combinations were PR*WC, PR*RC, C*RC and RC*P which had the highest fPARi (Figure 6.1).

The canopy structure of a species-mixed community is an important factor in determining the competitive ability of the plants to intercept light (Caldwell, 1987). The individual species' canopy contribution to light interception and competition for light is a natural phenomenon in a multiple-cropping system or multi-species pasture swards (Goudriaan and Monteith, 1990). The canopy fraction for light (PAR) interception of the sward is central to the growth of a pasture sward and consequently its biomass yield. This research did not measure the canopy properties (leaf angle, extinction coefficient) of the different mixes for light interception. However, it integrated their effect through measurement of the fPARi over time in Year 3. Results of light interception were consistent with those from biomass yield, highlighting the benefits of a perennial legume and grass mixture, specifically PR*WC and PR*RC. On average, the pasture species mixtures intercepted 17 MJ M^{-2} (7%) more PAR than the monocultures and provided 16% higher yield. Several previous studies have identified that species mixed stands have a denser canopy than monocultures because of the complementarity between species (Loreau and Hector, 2001; Tilman, 1999). Capture of light energy is the most important determinant of yield because light is instantaneous, if it is not captured immediately it is lost from the system.

Across the regrowth cycles, the average pre-grazing fraction of light interception of monocultures and species mixed pasture swards were 0.75-0.86 and 0.78 - 0.92, respectively. In addition, the fraction of light interception at the midpoint of each regrowth cycle was higher (0.49 to 0.70) for species mixed swards than the monocultures. This indicates that recovery from defoliation was more rapid than for the species mixed pasture swards. Several studies have confirmed that mixed cropping/diverse species produced a denser canopy than the monocultures (Sapijanskas et al., 2014). Canopy complementarity between species efficiently utilised growth resources (Loreau and Hector, 2001; Tilman, 1999), primarily light. The grazing practice used in this experiment allowed the monoculture sward canopy to be under optimum levels rather than the species mixed swards. Across the sward communities, the average fraction of light interception (> 0.90) at pre-grazing was higher in late spring (30/11/2020) and mid-summer (12/01/2021). The average cumulative intercepted light energy in the re-growth cycles was higher for mixture communities, which was consistent with the average biomass yields of 1.61 t/ha compared with the monocultures (1.45 t/ha). Radiation interception directly measures ground cover (Vos, 1995a), and quantifies the canopy potential for light interception (Wilson and MoGuire, 1961). Sollenberger (2018) considered 0.85 (fraction) light interception as a grazing benchmark. He stated that grazing is encouraged when the sward canopy reaches 0.8 to 0.9 fraction of light interception. This multi-species trial identified, on average, that 77% of sward communities (mono and binary mixture) reached more than 0.85 as a fraction of interception (pre-grazing) across the re-growth cycles. The fraction of light interception in spring and summer was 0.84 and 0.85, which was higher ($p<0.01$) than in autumn (0.78) under these irrigated conditions (Figure 6.2).

The analysis of fPARi over time highlighted when the positive interactions occurred. Specifically, in most cases for the spring rotations from September to January binary mix and component monocultures were not different in fPARi. However, in February and March the synergistic effect of having perennial ryegrass in the mix was apparent. For example, PR with P, WC or RC showed a positive response which was not apparent for the cocksfoot binary mixes. Also, the lowest cumulative PAR interception was in WC plots in these later rotations. By the third year, red clover can be expected to have lost its tap root and be producing dry matter based on water extraction from shallower nodal roots (Chapman et al., 1995a). The soil moisture deficit suggests that irrigation was sufficient to maintain leaf expansion in most species. However, the 80 mm of water applied in January (Table 6.2) may have been insufficient for the shallow rooted white clover.

The DI model quantified the additional fraction PAR interception, which represented the diversity contribution on canopy formation for PAR interception and RUE. The positive contribution of a binary mixture was found mainly for PR*WC and PR*RC. PR*WC intercepted a higher fraction of PAR than PR*RC. Light partitioning in an ecosystem or pasture ecosystem can be a subject mechanism of the diversity effect (Naeem et al., 1994). The interaction contribution for fraction PAR interception (additional canopy fraction) for the binary mixtures, PR*WC (0.47) and PR*RC (0.10), was further supported by improved biomass production of these swards. Thus, these results show the average interaction contribution for light interception in the 2020/21 production year reflects how those mixtures produced the most herbage biomass and was probably the same mechanism responsible for differences in 2018/19 and 2020/21.

These pasture species mixed swards improved canopy light interception and resulted in higher DM yield than monocultures, which is one of the goals of intercropping (Brooker et al., 2015b). In this experiment, biomass yield showed a positive linear relationship with intercepted light energy (Kiniry et al., 1989). Competition among species for light in diverse species pasture swards can facilitate an overinvest in light harvesting (Slattery and Ort, 2021), depending on the functional species. However, the higher biomass yield in these species mixed pasture swards was positively related to dense canopy cover (Spehn et al., 2005). The pasture growth rate is also related to the percentage of light intercepted by the canopy (Brougham, 1957). These results confirm the ecological theory that a positive diversity effect, through synergistic interaction for higher light interception and DM production, was observed in PR*WC and PR*RC binary mixtures. The RUE value of PR*WC and PR*RC were 1.55 and 1.67 g DM MJ^{-1}, and was enhanced particularly when compared with the grass monocultures. Specifically, these RUE values were higher than the proportional contribution of the component species in mixtures, of 1.32 and 1.29 g DM MJ^{-1}, respectively. The RUE value indirectly represents the net photosynthesis of swards from intercepted light energy. The DI model analysis for RUE quantified the contribution of component species on RUE of species mixed swards, mainly binary mixtures. The model provided the guideline for mixing species for improved biomass production efficiency. The appearance of some negative coefficient values suggests some reduction in relative abundance observed in those binary mixtures having the component species P and SC.

The RUE of perennial legumes was 1.69 g DM MJ^{-1} and was higher than the RUE of grass as a functional group (1.25 g DM MJ^{-1}). Among the monocultures, the lowest and highest biomass production efficiency per unit of intercepted light energy were observed for perennial ryegrass (1.32 g DM MJ^{-1}) and sub-clover (1.76 g DM MJ^{-1}). The RUE of cocksfoot was 1.19 g DM MJ^{-1}, association with RC (0.5:0.5) 1.35 g DM MJ^{-1}.

The differences in RUE of the mixture reflects canopy conversion efficiency which is affected by herbage N content, and weed proportion (Shewmaker and Hooper, 2007), and temperature (Justes et al., 2000). As a canopy component of the sward, weeds intercept light and contribute to biomass production, and the RUE calculation, which was inevitable in the experiment. The annual growth habit of SC was not appropriate for this experiment once the decision to irrigate was made. The annual death of the sub clover allowed space for immediate weed ingress and a rapid decline in the SC component after the first spring. The SC monoculture was 94% unsown species in the third year but had the highest RUE value of 1.76 g DM MJ^{-1}. The implication is that the weed species were successful because they had a comparatively high rate of photosynthesis across the season. This could be because the weeds were a combination of summer (e.g. fathen) and winter (e.g. chickweed) annuals. These combined species may have been able to exploit the seasonal changes in temperature more effectively than the sown perennials. This may reflect the emphasis of annual species on above ground dry matter and seed production compared with perennial species that also invest carbon in roots and storage organs to perennate. This difference in partitioning is not captured in traditional measures of RUE which are based only on above ground dry matter. To deal with these future studies on perennial pasture species may need to examine shoot and root growth, as was required to understand the growth and development of lucerne (Moot et al., 2003b).

Grass and clover associations improved the RUE of the mixture. For example, the RUE of PR*WC and PR*RC were 1.55 and 1.67 g DM MJ^{-1}, higher than the proportional contribution of monoculture species PR (1.31 g DM MJ^{-1}) and RC (1.56 g DM MJ^{-1}). This result is consistent with the legume component in binary mixtures improving the herbage/foliage N content. This could have improved photosynthesis (Mills et al., 2009; Peri et al., 2002; Sinclair and Horie, 1989) and therefore RUE (Teixeira et al., 2008). Higher N content improves leaf size, and canopy light interception (Bélanger et al., 1992). The mean N content of PR*WC (3.3%) and PR*RC (3.6%) were higher than that of the PR monoculture (2.6%), which showed obvious urine patches as a sign of nitrogen deficiency in the grass monocultures in Years 2 and 3.

Plate 6.1 N deficiency in PR monoculture plot, and strong growth of PR on urinated soil in a diverse species mixed pasture experiment at Lincoln University, Canterbury, New Zealand.

The effect of temperature on photosynthesis is important for plant species. Several research reports have explained the relationship between RUE and temperature for species such as lucerne (Brown et al., 2005) and maize (Andrade et al., 1993). In this experiment, the relationship between RUE and temperature was linear, as expected across this narrow temperature range (8 to 16 ˚C) which is in the linear response phase for C3, species which have an optimum of 20 to 25 ˚C (Sinclair and Muchow 1999). A positive response was found for all monocultures and selected binary mixtures such as PR*WC and PR*RC. The RUE and temperature relationship showed photosynthetic sensitivity to temperature ranged from 0.04 g DM MJ^{-1} $˚C^{-1}$ for C to 0.09 g DM MJ^{-1} $˚C^{-1}$ for P. Biomass production efficiency for unit temperature changes for monocultures such as PR, WC and SC were 0.05, 0.07 and 0.08 g DM MJ^{-1} $˚C^{-1}$, respectively. The RUE response to temperature changes in PR*WC and PR*RC were 0.05 g DM MJ^{-1} $˚C^{-1}$ and 0.08 g DM MJ^{-1} $˚C^{-1}$, respectively. Thus, these results show that changes in fPARi were the main cause of yield differences among pastures. However, differences in RUE also contributed positively to the performance of binary mixes that contained a grass and a legume due to the additional nitrogen available. In contrast increasing temperature had a consistent linear effect on RUE, but the starting value in each pasture differed with species and reflected their N level.

6.5 Conclusions

1. The species mixed pasture swards intercepted a higher fraction of PAR and had improved RUE which explained their higher herbage biomass yield compared with the monocultures.

2. Legume and non-legume mixtures improved the canopy light interception, producing higher biomass among the binary mixtures, particularly PR*WC and PR*RC.

3. Interspecific differences in RUE were observed between monocultures in Year 3. The perennial clovers and herbs produced more biomass per unit of intercepted light energy than the grass functional group, probably due to its lower herbage N content.

4. Monoculture species did not show differences in the RUE changes per unit temperature. Similarly, PR*WC and PR*RC also did not differ.

5. RUE of monoculture species differed and blending the species in binary combination improved the RUE value of mixtures depending on the functional group of species.

6. The mixture effect on canopy fraction light interception contributed more to the biomass production, and yield. Diversity contribution on RUE efficiency was also positive for mixture communities depending on the cooccurring functional species in the mixture.

Chapter 7

General discussion

The main aim of this thesis was to formulate a pasture seed mixture from a diverse pool of commonly used pasture species belonging to the functional groups of grasses (perennial ryegrass, cocksfoot), legumes (subterranean, red, and white clovers) and the herb plantain to exploit the diversity of productivity. The impact of the pasture diversity-productivity relationship in intensively managed pasture systems is a long-standing question, with the availability of many pasture plant species for seed mixture formulation. In diverse species mixed pasture, a seed mixture formulation that identifies the combination of species that are beneficial at the community level is a challenge for agronomists, even though pasture species characteristics are well documented (Charlton and Stewart, 2000). This experiment identified the pasture species and their optimal proportions in a seed mixture to achieve the main goal of the research, "optimal seed mixture formulation for improved productivity". The measured community responses were biomass yield, weed biomass yield, representing the weed suppressive effect and the main quality parameters- metabolizable energy and crude protein content.

This chapter discusses several key findings aligned with the objectives. It mainly considers the range of species and their relative proportion for mixtures, quantifying the mixture effect, persistence of community responses and optimisation of component species for improved responses. This was done through investigating the pasture swards' botanical composition dynamics over time. It also, identifies the physiological basis for improved productivity based on light interception and RUE, which was also influenced by temperature. The data used for analysis and reported in this thesis was obtained from 2018 to 2021 in an experiment which continued until 2023.

The analysis of the mixture experiment, based on the influence of species proportion on community responses provides new insights into the possible blending of pasture species combinations for improved productivity. In this experiment, the blending of common pasture species included perennial grasses, clovers and herbs. The experiment was changed from a dryland to irrigated experiment after sowing. This meant the use of subterranean clover was inappropriate. However, this treatment did provide the opportunity to examine the impact of weeds on the experiment. Furthermore, the simplex design is a powerful tool for quantifying interactions between the species in the multi-species pasture ecosystem, formulated from these six common pasture species. This is because the simplex design uses every combination of the species. This allows interactions to be detected and species proportions to be considered in greater detail than commonly occurs in pasture sowing rate experiments that may change the rate of one species (Example- perennial ryegrass) while holding the other species constant (Black, 2021). However, the simplex design resulted in 69 treatment combinations which when replicated created 276 plots to manage. Thus, a large component of the research was creating the appropriate statistical analyses with the regression analysis-based Response Surface Modelling (RSM) approach used. This identified the optimum proportion of the mixture components (PR, C, P, WC, RC and SC) to maximize the pasture responses. This approach eliminates the difficulties associated with traditional methods of competition studies, such as the replacement or additive series design (Connolly et al., 2001). The practical application of this research from a farmers' perspective is to determine how many species (functional species) and what relative proportion of component species in the seed mixture improve yield and quality over time. This experiment was performed with no nitrogen fertilizer treatment to utilize the biologically fixed N from the legume functional species in the mixture. Diversity tends to improve or maintain ecosystem services over time, particularly in less fertile soils (Hooper et al., 2005). In this experiment, no fertilizer application maintained a higher diversity over time (Gough et al., 2000; Mountford et al., 1993) and helped identify the mixture combination species. We only applied sulphur super 30, before starting the experiment as a soil amendment to maximise seedling growth.

Studying the functional species and blending them to bring out the diversity effect is the basis for pasture diversity experiments. Sanderson et al. (2004a) stated that mixing functional species improves biomass production primarily from legume and nonlegume species. The current experiment showed that diversity improved biomass productivity and created a positive yield from overyielding and transgressive over-yielding on average over 23 grazing cycles. Around 79% of pasture diversity experiments revealed that the pasture mixture community produced 1.7 times higher yield than their monocultures (Cardinale et al., 2007). In this experiment, species mixed swards gave, on average, 16% higher biomass yield and 64% lower weed biomass than the average for the monoculture species. This experiment identified that 2-3 species level mixture from legume and non-legume components in the mixture that provided the most consistent biomass yield over time with interactions between species. The lower biomass production of monocultures was due to the inability to utilize growth resources (Šidlauskaitė et al., 2022).

7.1 Diversity contribution to community responses

The quadratic and cubic models quantified the significant contribution of the mixing effect on biomass production and weed suppression. The DI model analysis for functional species identified that the grasses and perennial legumes produced the diversity and contributed to additional biomass yield. Further, the DI model for mixture component species identified that species proportions at two or three species levels significantly improved biomass yield and weed suppression over time. The interaction coefficient value for biomass production of mixture communities was positive, and the value was higher than 1 (>1) for PR*WC, PR*RC and C*RC binary (quadratic model, P=0.01) and ternary combinations; PR*WC*RC (cubic model, P=0.08). The significant interaction contribution for biomass production and weed suppression were higher for PR*WC, PR*RC and C*RC over the regrowth cycles. The diversity effect is the sum of all pairwise interactions in the mixture community (Black et al., 2017; Kirwan et al., 2009), responsible for the additional productivity of diverse species mixed pasture communities. The improved herbage production was a main concern in optimisation of the component species and the identified mixture communities produced 16 t/ha, from their interaction contribution. This interaction between species was for niche partitioning for growth resources and facilitated the utilization of N from the legume component (Andrews et al., 2011; Black et al., 2009; Connolly et al., 2011). The positive diversity effect could lead to higher stability of herbage biomass production (Haughey et al., 2018; Tilman et al., 2006). The higher coefficient value for PR*RC (1.64) and PR*WC*RC (4.14) indicated the additional interaction turnover of 0.41 t/ha (9.4 t/ha) and 0.11 t/ha (2.5 t/ha).

PR*WC under irrigated conditions in the Canterbury region has produced 1.9 to 2.5 t/ha in each grazing round (Clark et al., 2007). Yield of positive mixtures in this experiment was different under N fertilizer application. The mixing of species in this experiment identified overyielding and transgressive overyielding mixtures depending on the monoculture performance. The agronomically important transgressive overyielding mixtures were PR*WC (1.70 t/ha), PR*RC (1.90 t/ha), and C*RC (1.94 t/ha), which produced higher biomass than the best monoculture RC (1.67 t/ha). PR exhibited significant pairwise interactions with WC and RC for biomass production over the regrowth cycles (7 and 9 times in 23 regrowth cycles) in binary mixtures. In contrast, the WC*RC binary combination rarely (4 times in 23 regrowth cycles) showed a significant interaction during the experimental period.

The diversity effect primarily depends on the interaction between species (Black et al., 2018; Loreau and Hector, 2001; Trenbath, 1974), termed interspecific interaction. Pairwise interaction is the most common; the net effect is reflected as a community response. The three-way interaction in PR*WC*RC ternary mixture was at P=0.08, contributing to the mixture biomass productivity (1.86 t/ha). Moreover, the net effect of positive and negative pairwise interactions operating in the sward also provided a beneficial community response. A significant three-way interaction was observed for DM yield (refined cubic model) and weed suppression (cubic model), which rarely occurs between species (Black et al., 2021; Nyfeler et al., 2009a) in a ternary mixture.

The weed suppressive effect in a pasture sward is another important agronomic aspect. The significant quadratic and cubic models with higher negative coefficient values for weed biomass expressed the strong mixture effect in the pasture community. The negative direct interaction for weed biomass (suppressive effect) appears to be primarily caused by competition for growth resources such as light, water, and nutrients, which are often in limited supply (Goldberg, 1990; Miller and Travis, 1996). The negative coefficient value for weed suppression indicated that the weed biomass yield was lower than their monoculture effect.

The proportion of species in the seed mixture also significantly affected herbage nutritional composition (Deak et al., 2007), and species abundance played a role in quality improvement, particularly the contribution of legumes. Species composition from different functional groups has different chemical compositions and growth stages, and photosynthetic pathways affect the nutritional composition (Andueza et al., 2010; Čop et al., 2009). In a pasture mixture community, legumes, such as white clover and red clover, improve the nutritional value of herbage biomass (Sleugh et al., 2000) and palatability leading to preferential intake (Black et al., 2009). The interaction coefficient values for CP and ME were less than one and negative, indicating that the mixture effect was not dependent on diversity, but the identity effect was the main contributor to nutritional quality. For example, the CP content of PR was 16.8%, but combined with WC and RC produced herbage materials with higher CP content of 18.8% and 20.7%, respectively. This is consistent with previous findings that legumes in the mixture community improve the herbage quality. PR*WC, PR*RC and PR*WC*RC were the mixture combinations identified that offered the best diversity relationship to improve herbage biomass production with lower weed biomass and improved quality.

7.2 Mixture complexity

Pasture complexity makes it difficult to characterize a mixture of the community's biomass productivity and nutritional value. The ecosystem processes in this experiment were also complex because mixing one to six species from different functional species created a wide range of mixture communities. The simplex design with an associated RSM approach depicted the mixture effect in the wider community. Several studies have found no clear benefit of increasing the number of species in a pasture mixture community for yield stability (Sanderson, 2010). This experiment identified the strong diversity effect of combining key functional species (legume and non-legume), but only for 2 to 3 species. The mixture communities of PR*WC, PR*RC and PR*WC*RC maintained their ecosystem productivity over the experimental period. A similar finding was recorded in other pasture diversity experiments (Tilman et al., 2001). In addition to the mixture combinations identified in this experiment, other mixture combinations performed a secondary level of production responses, but these were inconsistent over time. Example- C*RC, C*WC, WC*P, PR*P and PR*C.

Species with a lower interaction coefficient value (< 1) express the competitive relationship, produce lower yields, and alter botanical compositions. Strong interactions in a pasture mixture community tend to remove or destabilise weak interactions fundamental to community diversity (Gellner and McCann, 2016). Properties of the interaction network also confirmed that when the diversity increases or number of terminals increases, the mean interaction strength decreases (Ushio, 2022). A significant interaction was observed at the 2 to 3 species level. The interaction between two species is usually altered by adding a third species to the system (Callaway and Pennings, 2000; Miller, 1994). Other studies on pasture agronomic systems also found that one or two species dominated the system (Kirwan et al., 2009). The optimal pasture seed mixture identified in this research indicated that a perennial grass and either 1 or 2 perennial legumes increased biomass productivity at 2 to 3 species level or simple mixture (Sanderson et al., 2004; Tracy and Sanderson, 2004). PR, WC and RC are the specific pasture mixture components commonly used in different climatic conditions and gave higher biomass yield than other mixture combinations.

7.3 Reasons for diversity contribution

Among the evaluated swards in this experiment, the identity contribution from both species in the binary mixtures was higher for C*RC (1.9 t/ha), PR*RC (2 t/ha) and PR*WC (1.7 t/ha). Further, the diversity contribution for additional biomass yield of these mixtures were 0.51, 0.41 and 0.29 t/ha, respectively. The equal relative abundance of the component species in the mixtures as formulated in this experiment, PR*RC and PR*WC, showed a potential for efficient light interception (Chapter 6) and herbage biomass production. DI modelling estimated the interaction contribution for the canopy fraction of interception for PR*WC and PR*RC were 0.47 and 0.10, respectively. The higher fraction of light interception in these mixtures (PR*WC and PR*RC) compared to their monocultures validates that the mixtures community improved light interception and biomass production. The main physiological reason is complementary leaf architecture which reduces the space in the canopy, so it intercepts more light energy (Spehn et al., 2005). PR and RC have erect growth habitats and WC has a prostrate growth habit. Differences in growth forms and leaf architecture contributed to higher canopy cover and light interception in PR*WC, PR*RC, and PR*WC*RC ternary mixture. The horizontal to vertical leaf arrangement of component species (Sonohat et al., 2002), improves the light interception potential for biomass production. This experiment did not directly investigate the canopy properties but evaluated the canopy potential for improved light interception and therefore biomass production. The positive interaction between species was a complementarity effect that enhanced production., For example, direct facilitation of N fixation from legumes and niche complementarity for growth resources with the canopy structure facilitated greater light capture. Interspecific variation between functional species (Tilman, 1996) and the presence of key species in the mixture affect the productivity and stability of the species mixed pasture swards (Grime, 1997; Huston, 1997). For example, in this experiment, greater biomass productivity was obtained from the component species PR (1.53 t/ha) and RC (1.67 t/ha). This may also have been aided by different root morphology. Species with contrasting deep and shallow roots can obtain greater soil occupation. This process enables mixtures to yield more than expected from their corresponding monoculture. Nutrient uptake of these mixtures also differed between them with respect to time and type or form (Weigelt et al., 2005). It is also likely that in this grazed situation the N supply from the legume improved the mixture performance (Andrews et al., 2011; Jumpponen et al., 2002). Deak et al. (2007) found that the CP concentration of the pasture mixture was dependent on the proportion of legume. The grass (PR) based mixture communities having RC and WC produced consistent higher biomass yield over time. In this experiment, monoculture yield of PR and C reduced, and perennial clovers (WC/RC) maintained the production over time, but the binary mixture of PR*WC and PR*RC increased over time.   reduced Grass-legume mixture can yield more nitrogen than a legume monoculture due to the provision of N from symbiotic and non-symbiotic relationships (Nyfeler et al., 2011). For example, the CP content of PR and RC were 16.8% and 24.4%, but both species in the binary mixture (0.5:0.5) which produced a herbage biomass yield with CP content of 20.7%. It is well known that grass-legume mixtures improve the nutritional value of herbage biomass. This is because of differences in the chemical composition and digestibility of the species (Duru et al., 2008). The improved biomass yield and nutritional value of the identified optimal mixture combinations such as PR*RC and PR*WC*RC was mainly due to the blending of productive species from legume and non-legume functional species and diversity contribution/interaction contribution.

7.4 Pasture persistence

A key component of pastures for on-farm performance is their persistence. Mixture combinations need to have sufficient stability and persistence (Finn et al., 2013b). This was analysed in different ways including the significant interaction with higher coefficient value over the regrowth cycles, and the RGR of swards compared annually (1st to 2nd year and 2nd to 3rd year). RGRD value comparisons for binary mixtures and botanical composition ratio quantifies the sown species compositional changes over the years. The analysis of the interaction patterns across several regrowth cycles showed several significant interactions with higher coefficient values, in several re-growth cycles for PR*WC and PR*RC mixture communities (PR*WC, 7 times and PR*RC, 9 times out of 23 regrowth cycles), with improved herbage biomass yield (mixture effect) over time. The identified mixture communities such as PR*WC, PR*RC and PR*WC*RC maintained the productivity for four years, which is an indication that these communities were still productive and may maintain their sward responses beyond the duration of this experiment. The positive diversity effect over the regrowth cycle for pasture responses (herbage biomass) provides greater stability of these sward responses over time (Haughey et al., 2018; Tilman et al., 2006). In contrast to the positive coefficient value for biomass production, the weed suppressive effect denoted by a negative value means suppression of the weed biomass yield at levels lower than their monocultures. C*RC was another productive binary mixture with higher significant interaction coefficient values over the regrowth cycles. Positive interaction or facilitation enhances the growth, survival and reproductive performance of associated plants or co-occurring species (Callaway and Pennings, 2000) and facilitates the beneficial diversity effect over time. Response stability was evaluated based on the biomass proportion of the component even though there was an equal number of plants of each species in the mixture, giving a total plant density of 1000 plants/m^2. PR*WC and PR*RC binary mixtures maintained the highest sown species proportion of 0.92-0.96 and 0.88 to 0.92 over three years. This was the result of their significant suppression of un-sown species identified through quadratic modelling for weed biomass. The significant interaction with higher negative coefficient values for weed biomass in PR*WC and PR*RC were 21 and 16 times over the 23 growth cycles. This result confirms that this mixture community persisted over the years of the experiment.

The RGR value comparison of monoculture species over the years (1st to 2nd year; 2nd to 3rd year) revealed that the grass had a greater potential with a different degree of positive biomass production than the clover components. Biomass production of WC increased in 1st to 2nd year and then reduced from 2nd to 3rd year. RC and P biomass productivity reduced from 2nd to 3rd year. The net relative growth rate of the component species in the mixtures showed positive and negative values depending on the component species in the mixture. The biomass production potential of PR*WC, PR*RC and PR*WC*RC in the first comparison period (1st to 2nd year) was 0.30 $tt^{-1}ha^{-1}$, 0.24 $tt^{-1}ha^{-1}$ and – 0.03 $tt^{-1}ha^{-1}$, respectively. Those mixtures in the second comparison period (2nd to 3rd year) were -0.03 $tt^{-1}ha^{-1}$, 0.10 $tt^{-1}ha^{-1}$ and – 0.03 $tt^{-1}ha^{-1}$. PR*WC*RC maintained biomass productivity with fewer changes in biomass production. The RGR comparison for monoculture species did not explain the changes in botanical composition.

7.5 Botanical composition

Changes in botanical composition occurred continuously over time and deviated from the initial sown proportion which is expected (Fridley et al., 2007). RGRD value comparisons of binary mixtures showed different degrees of compositional changes relevant to the first or second species in the mixture (Connolly and Wayne, 2005; Suter et al., 2007), dominating or diminishing the component species in the mixture. The expectation is that the mixture's higher growth rate of co-occurring species for more biomass yield and lower RGRD value between species maintain the botanical composition. The identified mixtures, such as PR*RC maintained the composition over the years. PR*WC binary mixture maintained its composition from 2nd to 3rd year, even though PR dominated in the first year. The susceptible binary mixtures for compositional changes were C*WC and C*P, where C dominated overtime.

The significant interaction between component species with higher coefficient values reflected the production stability. Grass generally resists the invading species into the system more than legumes (Mwangi et al., 2007), thus maintaining persistence. In legume and non-legume mixtures, legume proportions fluctuate from low to high levels, but maintaining 1/3 of the legume in the mixed swards system fulfils the system's N requirement or provides maximum N yield (Suter et al., 2015). The availability of nitrogen creates a predator prey situation in which the grass and clover have periods of dominance (Chapman et al., 1995b).

The analysis of the botanical composition ratio in the mixtures showed minimal changes in the PR or C-based 2 or 3-species mixture community, which included WC or RC. Clover component proportion fluctuated over time in binary and ternary mixtures in association with grass. In general, the prostrate growth habit of white clover competes for and seeks out areas of high light in PR swards which in turn leads to a lower percentage of it in the biomass of the sward (Stewart et al., 2014). Pasture species mixture studies revealed that, compared with the perennial ryegrass monoculture, the PR-based mixture with legume (PR, WC) sustained the herbage yield (Kirwan et al., 2007; Wilsey and Potvin, 2000). Legume components in the mixture generally compensate for the grass's lower growth rate in summer or for seasonal yield reduction (Sleugh et al., 2000). The biomass production stability of PR*WC, PR*RC and PR*WC*RC was a result of maintaining the legume proportion over the regrowth cycles. (Schwinning and Parsons, 1996) found that grass-legume associations are intrinsically unstable.

Sanderson et al. (2012) stated that 30 to 40% of legume content in a diverse species mixed pasture sward is considered as an optimum level. This guideline can be used to assess the persistence of a mixture composition. PR*RC binary mixture maintained the RC proportion, ranging from 0.29 to 0.41. In PR*WC*RC ternary mixture, WC and RC as perennial legume functional component species maintained the legume biomass yield proportions of 0.38, 0.31 and 0.46 in each year in a sequence from the establishment year, and thus met that optimal proportion.

Modelling RGRD values to the sown proportion revealed that the equal proportion of PR, WC and RC as an equal number of seeds per species, at a relative abundance of 1000 plants per square meter, maintained the component species proportion with minimal changes.

The major causes of the botanical composition changes are competition (Goldberg, 1990; Goldberg and Barton, 1992) and climatic factors (Callaway and Walker, 1997). WC in the binary and ternary mixtures showed a reducing trend in the second year but increased in the third year. WC is temporarily more persistent than RC (Stewart et al., 2014), but a lower proportion of WC in the mixture can be expected due to its poor competitive ability and propagation in the establishment phase (Lane et al., 2000). However, its stoloniferous growth over time improves persistence (Black et al., 2017). PR*WC*RC and PR*RC mixture communities maintain the RC proportion of 0.31 to 0.33 for up to three years. From a pasture mixture experiment, Thomas (1992) found that 0.20 to 0.50 proportion of biomass yield was from legume, indicating the positive diversity in the pasture mixture community (Brophy et al., 2017). Black et al. (2009) stated that maintaining RC in the pasture mixture after the 3rd year is challenging in pasture management. The loss of the legume component was observed in several mixture combinations in this experiment. SC did not persist in the mixture after the establishment cycle because the pastures were irrigated. In summer dry rainfed situations cocksfoot and the annual clover sward mix is most stable and the reduction in the clover component can be managed through grazing (Mills et al., 2015; Monks et al., 2008). When there is a reduction in the legume content of the herbage, there is also a reduction in herbage quality and animal production (Reed, 1981). PR*WC, PR*RC and PR*WC*RC mixtures were identified as persistent mixture communities that maintained the botanical composition and productivity.

This research also quantified the effect of light energy on compositional changes of binary mixtures. The species proportion dynamics are generally a combined effect of the interaction between species and the environment (Chapman et al., 2011), quantified in a regression prediction analysis. Grasses (PR or C) positively influenced RGRD values in contrast to perennial legumes, which were either positive or negative depending on species, soil N, or climatic factors. A turf grass botanical composition study revealed that competition for light is the major factor in modifying species proportions (Watkins, 2022). Light also affects the understory plant diversity in forestry (Bartels and Chen, 2010). The higher positive and negative coefficient values for RGR indicated that the botanical composition in this experiment was primarily driven by the RGR of individual species in the mixture combination. The cause of differences in RGR may be due to differences in the leaf area of individual species which affects their ability to intercept light, but this was not examined in detail in this study.

7.6 Light interception

In this experiment, higher canopy light interception and intercepted energy of species mixed pasture swards produced greater herbage biomass yield than the monoculture species. This finding confirms general rules that the diverse species pasture system uses resources efficiently, most notably a higher canopy fraction of light interception, associated with increased total photosynthesis and consequently DM production. There was a blending effect of species in binary mixture on light interception between species, showing increased or decreased interaction contributions. Positive coefficient values for the fraction of light interception indicated the canopy contribution for the higher light interception, was dominant in C*RC, PR*WC, PR*RC and C*RC (Appendix P). In most cases, species in the mixed swards with different leaf arrangements due to the functional species, allows greater light interception for growth and DM production (Black et al., 2009; Hay and Porter, 2006). Radiation interception of a canopy directly represents the ground cover fraction (Vos, 1995a) and can be quantified to assess canopy potential (Wilson and MoGuire, 1961). The mixed-species strand had a denser canopy than the monoculture due to the complementary canopy architecture and plasticity (Pretzsch, 2014). In this experiment, species pasture swards intercepted more canopy fPARi than monocultures. In a multispecies experiment the optimum grazing time is obtained at different times. Thus, the management of the experiment may have adversely affected some of the species' combinations that were slower to recover from grazing. In this experiment, legume: legume and legume: herb mixtures took longer to develop the canopy than grass : legume mixtures which formed a canopy more quickly and intercepted more light so produced more herbage biomass. General regrowth interval/grazing round applicable based on both farmers' practice and targeted pre-grazing biomass yield may not be appropriate for some pasture sward communities, particularly clover or plantain monocultures. This experiment provided an opportunity to screen how the higher diversity contributed to leading mixtures, even though some communities are under sub optimal production which is unavoidable in large scale mixture experiments.

The results showed that the PR*WC and PR*RC intercepted a higher fraction of light in both the middle of the re-growth cycle and the pre-grazing stage. Several mixture combinations were identified for their higher fraction of light interception and improved biomass productivity, namely PR*WC, PR*RC, and C*RC. The primary productivity in species mixed swards with design species relied primarily on light interception. PR and RC showed less competition for light than WC due to the erect growth habit (Stewart et al., 2014). These species both have the potential to suppress the WC.

The PR*RC canopy intercepted more light energy than PR*WC among the identified mixtures. The mixture trend in canopy light interception was given in positive and negative coefficient values (Appendix P). The positive coefficient values indicate a canopy complementary effect (Sapijanskas et al., 2014) for light interception and negative values are associated with a reduction of sown species in the community. The expectation of blending species was that canopy expansion of diverse pasture swards would exceed the monoculture component species and improve photo-assimilation and biomass production. The analysis in the third year showed that differences in canopy light interception mainly occurred in the latter part of the season when the white clover may have been compromised by shallow roots which restricted leaf area expansion. This suggests that more frequent irrigation events may be required to ensure it is not under moisture stress in a mixed sward.

Managing the sward canopy for efficient light interception is the main focus in designing the pasture mixture, as the biomass yield of pasture sward is proportional to the light energy intercepted (Moot et al., 2021a). Blending the pasture species modifies the sward canopy for radiation interception and light spectral changes within the canopy (Russell et al., 1989), thereby improving biomass production. In addition, the microclimate is modified in the diverse mixture system, especially the light regime (Tournebize and Sinoquet, 1995). Light interception measurements that include both canopy of weeds and pasture species are unavoidable in light interception measurement.

The differences in RUE were observed between species (interspecific variation), and mixture communities and differed with season. Six binary mixture communities had higher RUE including PR*WC and PR*RC. Blending legume and non-legume (PR*WC, PR*RC, C*RC) improved the biomass production due to the adding of N into the system. The herbage N content indicated that the addition of a legume to the grass increased the RUE, which is expected from greater rubisco per unit leaf area. Temperature is another important factor affecting crops' photosynthesis and RUE (Monteith, 1972). The average temperature in the 2020/2021 season during the light interception measurement ranged from 8 to 16 ˚C, which is in the range when a linear increase in photosynthesis is expected with increased temperature (Black et al., 2006; Moot et al., 2000). Temperature changes influenced the RUE of monocultures and binary mixtures consistently across treatments. The weed content of herbage also affected RUE, with the shallow rooted annual weeds producing high RUE values most likely because they invested more carbon in shoots than perennial species.

7.7 Factors influencing this mixture experiment: soil moisture, temperature and weed fraction.

The grass functional component had a lower RUE which was increased in the grass-clover binary mixture. For example, the RUE of PR and C were 1.32 and 1.19 g DM MJ^{-1}, respectively. N content of PR and C herbage was 2.6 and 2.7%, respectively. When these component species were sown with RC at 0.5:0.5 the herbage biomass N content averaged 3.6% (PR*RC) and 3.5% (C*RC). This resulted in RUE for PR*RC and C*RC of 1.68 g DM MJ^{-1} and 1.35 g DM MJ^{-1}, respectively. N levels influence leaf emergence, expansion, and duration, which increase light interception and photosynthesis (Mills et al., 2009; Novoa and Loomis, 1981; Peri et al., 2002). In C*RC pasture mixture, the RC, which fixes atmospheric nitrogen and improved the RUE. The increased N content in cocksfoot resulted in more PAR interception (Baker and Younger, 1987) and produced more biomass yield. The higher proportion of RC in a pasture sward generally fixes more N at 200 kg N/tons of legume/ha (Black et al., 2009) providing N to the associated species, predominantly through grazing. The N content of the PR*RC binary mixture sward was 3.6% in 2020/21.

Temperature influenced the RUE of monoculture swards, ranging from 0.04 g DM MJ^{-1} ˚C^{-1} in C to 0.09 g DM MJ^{-1} ˚C^{-1} in P. The temperature effect on monoculture swards differed (P=0.05) among species but did not differ from the binary mixtures such as PR*WC and PR*RC.

The above-ground herbage materials included the weeds fraction, which was unavoidable in the calculation of RUE as an integrated part of photosynthetic biomass. VWC in unsown species proportion added N to the system, and improved photosynthesis, and RUE. Diversity in weed suppression was significant and strong in this multi-species trial, and weed biomass was reduced with species richness. One expected benefit of multi-species pasture farming is suppressing weed growth. Interaction between species in the mixture community suppresses weed growth due to the creation of a competitive environment (Aarssen, 1983). The annual average unsown species proportion of binary and ternary mixtures were 0.26 and 0.15, respectively. The interference of weed on diversity contribution was not considered in this research. The weed species in the experiment were from different families which showed different growth and morphology characteristics. The common weeds were annuals poa (*Poa annua* L.), chick weed (*Stellaria media* L.), fathen (*Chenopodium album* L.), wire weed (*polygonum aviculare* L.) cleavers (Galium aparine L.) and willow weed (*persicaria maculosa* L). The perennial weeds were dock (*Rumex obtusifolius* L.) and dandelion species (*Tarraxacum mongolicum* L). The PR*WC and PR*RC mixtures had lower unsown species fraction of 0.05 and 0.12, respectively.

The soil moisture deficit was calculated based on PET, irrigation, and rainfall. The soil moisture deficit (ASMD/PSMD) of the experimental site is presented Figure 3.1. Irrigation was carried out when the soil moisture deficit reached 50 to 72% of the PAWC which aligns with general recommendations to irrigate when the soil moisture deficit reaches 50% of available soil moisture (DairyNZ, 2023). The monthly soil moisture balance calculation and soil moisture deficit of the monocultures showed the seasonal water deficit increased in February, March, and April each year, but did not exceed 72% of the PAWC. Irrigation maintained soil water levels above those expected to cause a reduction in yield, particularly for the deep-rooted species. The RC monoculture sward reached the soil moisture deficit level of 65 to 69% of field capacity during mid-summer (03-06-2019, 22-02-2021, 18-03-2021 and 06-09-2021). Similarly, WC experienced 65 and 66% deficit on 21-02-2020 and 03-05-2020. However, the WC plots had become invaded by weeds by Year 3 when the light interception measurements were taken. It is likely that the WC tap roots were gone by this time (Widdup, 2003) and the water extraction measurements were recording the extraction by weeds (Figure 6.7).

7.8 Optimisation of component species

The fitted model identified that PR*RC (0.5 : 0.5=12.6 : 13.4 kg/ha), and PR*WC*RC mixtures in two proportional settings mixture components (45 : 11 :4 4 and 50 : 15 : 35=11.3 : 0.8 : 11.8 kg/ha and 12.6 : 1.0 : 9.4 Kg/ha) maximized pasture responses. These mixtures consisted of functional components from grass and legumes. PR was the dominant species, herbage proportion in the seed blend ranging from 0.45 to 0.50 in all identified mixture combinations. The greater proportion of PR in the mixture contributed more to biomass yield, and RC improved CP content (Deak et al., 2007). The binary mixtures such as PR*WC, PR*RC and the ternary mixture PR*WC*RC maintained the PR proportion between 0.49 and 0.83. The productivity and stability of the ecosystem do not depend on the number of species. However, the key species (PR, RC, or WC) from the respective functional group is important (Grime, 1997). Cocksfoot (C) and red clover binary mixture was another identified binary mixture that gave higher biomass yield in this experiment but was not included in the optimisation process. Despite being the second most commonly cultivated species in New Zealand (Barker et al., 1985) cocksfoot shows major constraints such as low palatability, higher susceptibility to rust, poor persistence under sheep grazing (Moloney, 1993) and suppression of white clover or companion grown in medium to high fertile lowlands (Sangakkara et al., 1982).

Balancing the WC and RC population with PR, as identified in the optimal mixture, indicated a good scope for maintaining the desired proportion based on their functional species. The higher monoculture coefficient value (PR-1.65, RC-1.69, WC- 1.44), higher interaction coefficient value of 4.4 for PR*WC*RC (cubic model) and the maintenance of higher coefficient values (quadratic model) in PR*WC and PR*RC over the regrowth cycles confirms the stability of the biomass production over growing seasons (Sanderson et al., 2016; Sleugh et al., 2000).

Pasture seed mixture formulation for the establishment of multi-species pastures in an intensive farming system is an important decision-support tool that can be used in pastoral farming (Moot et al., 2021b). The pasture mixture optimization experiment identified that a two species mixture consisting of 60% Italian ryegrass and 40% red clover produced a higher yield (Ryan-Salter and Black, 2012). This result confirmed our findings as the PR fraction was higher than the RC proportion. RC improved biomass yield in association with PR. RC is a tap-rooted perennial legume for dryland regions. PR*WC is a long-term persistent species in nature, adapted to the temperature range of 10 - 20 ˚C under no moisture stress conditions (Chapman et al., 2011). The PR*WC pair in the ternary mixture (PR*WC*RC) contributed significantly ($P < 0.08$) to the mixture effect. The selection of species in the mixture maintained the interaction in such a way as to keep the balance of species over time (Hill, 1990; Lüscher et al., 1992), observed in PR*WC, PR*RC and PR*WC*RC mixture communities.

Legumes can improve the nutritional value of forage mixtures because of their high CP content and digestibility (Van Soest, 1987), as was observed in this experiment. Those species (legume and non-legume) mixed pasture ecosystem processes contributed to the sward community responses (Black and Lucas, 2018; Brophy et al., 2017; Connolly et al., 2009; Finn et al., 2013b; Kirwan et al., 2014). In this study, herbage biomass yield and weed suppression was achieved from the contribution of identity and interaction, but only identity contributed to nutritional composition. Optimisation of mixture communities ranging from 0.45 to 0.50 PR is expected to maintain the botanical composition, yield, and quality over time. Comparing all mixture communities for persistence improved production through diversity and was achieved in the PR*RC (0.5:0.5) binary mixture and expected from optimal ternary mixture PR*WC*RC (45:11:44 and 50:15:35). On average these mixtures produced 16 t/ha/yr (1.95 to 1.98 t/ha) herbage biomass from the experimental site. In 1998 to 2001, at this experimental site chicory and red clover monoculture produced an average annual herbage biomass yield of 15 t/ha and lucerne gave the highest biomass yield (20 t/ha). These species in the above mixtures maintained the component species proportion. The equilibrium of the above species avoids competitive exclusion. The diversity contribution of the above mixtures was due to the interspecific interaction between species and a higher relative proportion of component species PR. Combining the species identified in the experiment with relative proportion utilizes the growth resources from their niche or niche separation (Hill, 1990), balancing the competitive ability and the species proportions in the mixture.

7.9 Implementation in pastoral farming

This long-term (more than three years) study confirmed that cultivating the pasture species as monoculture is not advisable because it inefficiently utilizes the growth resources and produces herbage biomass with inferior quality due to increased weed content. The mixture of species from different functional groups (legume/non-legume) improved the production over time without applying N fertilizer. Grass (PR) with perennial clovers (WC/RC) brings out the positive effect of community responses- yield and weed suppression. The identified mixture communities were PR:RC at the ratio of 0.5:0.5 (12.6 : 13.4 Kg/ha); PR: WC:RC in both 45:11:44 (11.3 : 0.8 : 11.8 kg/ha) and 50:15:35 (12.6 : 1.0 : 9.4 kg/ha). A relative abundance of 1000 plants/m^2 improved the herbage biomass yield with less weed fraction and maintained quality over three years. These mixture communities will contribute to the animal feed in NZ, increasing primary productivity and quality of mixtures, which will significantly impact pastoral farming. These mixture communities maintain diversity-productivity and diversity-stability over three years.

7.10 Future research

1. The key to manipulating pasture mixture for long-term sustainable production is to understand the botanical composition of the mixture. PR-based binary and ternary mixtures such as PR*RC and PR*WC*RC were identified as the best-performing mixture communities. The beneficial effect was from identity and the interaction between these species. The interaction capacity of this mixture begins with an equal proportion of species (number of species) in the mixture, but the RSM procedure optimised the component species proportions and identified the best combination of species over time. The question is how the optimal component maintains the composition. The optimal pasture seed mixtures identified in this research evaluated for yield and quality and persistence overtime. The yield and quality persistence of optimal mixture community PR*WC*RC needs to be evaluated in different environment for sustainable production, beyond the four years of this experiment.

2. Further research to explore the diversity effect in this area should include more species, different soil types or cultivation practices and rainfed conditions.

3. Grazing studies suggest that the best time to graze is when there is 80 to 90% fraction of light interception (Sollenberger, 2018). This practice allows forage quality to be maintained as stated in the NZ guide; grazing at the 2 to 3-leaf stage provides higher quality herbage materials and improves herbage growth and animal production (David et al., 2016), which needs to be explored in the future.

4. A study on multi-species sward canopy architecture is essential to determine the contribution of the component species to the canopy pack for efficient light interception and light spectral changes in the canopy (Marshall and Willey, 1983).

5. Stock preferential grazing or grazing palatable species in swards allows unpalatable species to dominate in the swards. The botanical composition shifting pattern under preferential grazing needs to be investigated.

7.11 Conclusion

PR, WC and RC were the identified key species that improved the productivity and stability of the swards. The optimal proportion of component species in the identified pasture mixtures (PR*WC, PR*RC, and PR*WC*RC) produced more biomass yield with less weed fraction than other combinations over time. Nutritional quality (CP and ME) was improved mainly from the identity contribution in the mixtures rather than diversity contribution. Overall, pasture mixtures showed a clear advantage over monocultures. The study identified different mixture combinations and their community responses for major pasture properties of yield, weed suppressive effect and quality parameters. Some mixture combinations were inferior to the identified mixtures. The greater absolute pasture responses with greater diversity may be restricted to the choice of the species combination, relative abundance, and environmental condition (based on our study light temperature). The more productive or competitive species in the identified mixture (PR*WC and PR*RC) proportions translated to their overall average performance. This confirmed the component species (PR*WC*RC), formed an efficient canopy that intercepts more PAR energy due to diversity contribution. The stability of the mixtures was quantified based on pasture response and interaction between component species and suggests that they maintained their diversity and productivity over time as shown by acceptable botanical composition. This productivity and stability of mixtures were mostly comparable to the generally cultivated pasture mixture performance under fertilizer application. The mixture stability suggests that there was no need to apply N fertilizer. The higher interaction coefficient represents the strength of the diversity effect, which was contribution of legumes, even in low richness (2-3 species level).

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List of Appendix

Appendix A Cubic model analysis output for an average of three years biomass yield (Šidlauskaitė et al.), model summary and associated ANOVA

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.1258	0.0196	6.42	0.000	1.50
2	0.0200	0.0196	1.02	0.308	1.50
3	-0.1403	0.0196	-7.16	0.000	1.50
PR	1.5801	0.0919	*	*	4.39
C	1.2221	0.0919	*	*	4.39
P	1.5594	0.0919	*	*	4.39
WC	1.4268	0.0919	*	*	4.39
RC	1.6946	0.0919	*	*	4.39
SC	1.4086	0.0919	*	*	4.39
PR*C	0.444	0.450	0.99	0.325	3.38
PR*P	0.481	0.450	1.07	0.286	3.38
PR*WC	0.716	0.450	1.59	0.113	3.38
PR*RC	1.008	0.450	2.24	0.026	3.38
PR*SC	0.777	0.450	1.73	0.085	3.38
C*P	0.515	0.450	1.14	0.254	3.38
C*WC	0.605	0.450	1.35	0.180	3.38
C*RC	1.921	0.450	4.27	0.000	3.38
C*SC	-0.120	0.450	-0.27	0.789	3.38
P*WC	0.019	0.450	0.04	0.966	3.38
P*RC	-0.384	0.450	-0.85	0.394	3.38
P*SC	-0.453	0.450	-1.01	0.315	3.38
WC*RC	0.211	0.450	0.47	0.639	3.38
WC*SC	-0.019	0.450	-0.04	0.966	3.38
RC*SC	0.034	0.450	0.07	0.940	3.38
PR*C*P	2.32	2.64	0.88	0.382	1.88
PR*C*WC	1.32	2.64	0.50	0.618	1.88
PR*C*RC	0.61	2.64	0.23	0.816	1.88
PR*C*SC	1.62	2.64	0.61	0.539	1.88
PR*P*WC	0.34	2.64	0.13	0.897	1.88
PR*P*RC	3.36	2.64	1.27	0.205	1.88
PR*P*SC	0.98	2.64	0.37	0.712	1.88
PR*WC*RC	3.32	2.64	1.26	0.210	1.88
PR*WC*SC	4.33	2.64	1.64	0.102	1.88
PR*RC*SC	4.79	2.64	1.81	0.071	1.88
C*P*WC	-0.29	2.64	-0.11	0.913	1.88
C*P*RC	-1.05	2.64	-0.40	0.691	1.88
C*P*SC	4.07	2.64	1.54	0.125	1.88
C*WC*RC	2.34	2.64	0.88	0.378	1.88
C*WC*SC	3.17	2.64	1.20	0.232	1.88
C*RC*SC	0.91	2.64	0.34	0.731	1.88
P*WC*RC	5.08	2.64	1.92	0.056	1.88
P*WC*SC	5.05	2.64	1.91	0.057	1.88
P*RC*SC	3.49	2.64	1.32	0.187	1.88
WC*RC*SC	0.46	2.64	0.18	0.861	1.88

Model summary of quadratic model for herbage biomass (Šidlauskaitė et al.)

S	R-sq	R-sq[adj]	PRESS	R-sq[pred]
0.187871	54.14%	45.64%	11.4593	35.82%

Analysis of Variance of quadratic model analysis for herbage biomass yield (Šidlauskaitė et al.) (component proportion)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	2.4807	2.48074	0.826912	23.43	0.000
Regression	40	7.1855	7.18551	0.179638	5.09	0.000
Linear	5	2.9948	0.57441	0.114882	3.25	0.007
Quadratic	15	3.3963	1.12561	0.075041	2.13	0.010
PR*C	1	0.0173	0.03435	0.034350	0.97	0.325
PR*P	1	0.0956	0.04031	0.040312	1.14	0.286
PR*WC	1	0.2416	0.08950	0.089504	2.54	0.113
PR*RC	1	0.5910	0.17738	0.177384	5.03	0.026
PR*SC	1	0.5764	0.10548	0.105476	2.99	0.085
C*P	1	0.0901	0.04622	0.046216	1.31	0.254
C*WC	1	0.1367	0.06386	0.063861	1.81	0.180
C*RC	1	1.1860	0.64409	0.644091	18.25	0.000
C*SC	1	0.0321	0.00253	0.002527	0.07	0.789
P*WC	1	0.0587	0.00007	0.000066	0.00	0.966
P*RC	1	0.0026	0.02578	0.025778	0.73	0.394
P*SC	1	0.0116	0.03576	0.035765	1.01	0.315
WC*RC	1	0.1556	0.00776	0.007764	0.22	0.639
WC*SC	1	0.1170	0.00006	0.000063	0.00	0.966
RC*SC	1	0.0839	0.00020	0.000197	0.01	0.940
Special Cubic	20	0.7944	0.79438	0.039719	1.13	0.324
PR*C*P	1	0.0245	0.02707	0.027074	0.77	0.382
PR*C*WC	1	0.0057	0.00879	0.008787	0.25	0.618
PR*C*RC	1	0.0006	0.00191	0.001908	0.05	0.816
PR*C*SC	1	0.0081	0.01334	0.013336	0.38	0.539
PR*P*WC	1	0.0000	0.00059	0.000587	0.02	0.897
PR*P*RC	1	0.0467	0.05693	0.056934	1.61	0.205
PR*P*SC	1	0.0013	0.00483	0.004835	0.14	0.712
PR*WC*RC	1	0.0445	0.05577	0.055772	1.58	0.210
PR*WC*SC	1	0.0838	0.09490	0.094902	2.69	0.102
PR*RC*SC	1	0.1137	0.11607	0.116069	3.29	0.071
C*P*WC	1	0.0024	0.00042	0.000419	0.01	0.913
C*P*RC	1	0.0097	0.00558	0.005579	0.16	0.691
C*P*SC	1	0.0730	0.08348	0.083480	2.37	0.125
C*WC*RC	1	0.0232	0.02754	0.027538	0.78	0.378
C*WC*SC	1	0.0469	0.05062	0.050620	1.43	0.232
C*RC*SC	1	0.0037	0.00418	0.004181	0.12	0.731
P*WC*RC	1	0.1201	0.13040	0.130395	3.69	0.056
P*WC*SC	1	0.1241	0.12869	0.128687	3.65	0.057
P*RC*SC	1	0.0613	0.06168	0.061684	1.75	0.187
WC*RC*SC	1	0.0011	0.00108	0.001083	0.03	0.861
Residual Error	232	8.1886	8.18857	0.035296		
Total	275	17.8548				

Appendix B Refined cubic model analysis output for an average of three years biomass yield (Šidlauskaitė et al.), model summary and associated ANOVA.

Term	**Coef**	**SE Coef**	**T-Value**	**P-Value**	**VIF**
Blocks					
1	0.1258	0.0194	6.49	0.000	1.50
2	0.0200	0.0194	1.03	0.303	1.50
3	-0.1403	0.0194	-7.24	0.000	1.50
PR	1.6568	0.0743	*	*	2.93
C	1.3054	0.0656	*	*	2.29
P	1.5952	0.0534	*	*	1.52
WC	1.4404	0.0671	*	*	2.39
RC	1.6998	0.0661	*	*	2.32
SC	1.4251	0.0589	*	*	1.84
PR*WC	0.664	0.377	1.76	0.080	2.42
PR*RC	1.069	0.377	2.84	0.005	2.42
PR*SC	0.766	0.376	2.03	0.043	2.42
C*WC	0.856	0.333	2.57	0.011	1.89
C*RC	1.962	0.333	5.90	0.000	1.89
PR*C*P	6.08	2.16	2.82	0.005	1.27
PR*WC*RC	4.14	2.42	1.71	0.088	1.60
PR*WC*SC	4.55	2.42	1.88	0.061	1.60
PR*RC*SC	5.27	2.41	2.19	0.029	1.59
P*WC*RC	5.20	2.17	2.39	0.017	1.29
P*WC*SC	4.74	2.17	2.19	0.030	1.29

Cubic model summary for YT

S	**R-sq**	**R-sq[adj)**	**PRESS**	**R-sq[pred]**
0.186000	50.40%	46.72%	10.2550	42.56%

Analysis of Variance of cubic model for herbage biomass yield (Šidlauskaitė et al.) (component proportion)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	2.4807	2.4807	0.82691	23.90	0.000
Regression	16	6.5175	6.5175	0.40735	11.77	0.000
Linear	5	2.9948	0.9317	0.18635	5.39	0.000
Quadratic	5	2.4652	1.7734	0.35467	10.25	0.000
PR*WC	1	0.2121	0.1071	0.10710	3.10	0.080
PR*RC	1	0.5327	0.2786	0.27863	8.05	0.005
PR*SC	1	0.5032	0.1432	0.14322	4.14	0.043
C*WC	1	0.1153	0.2286	0.22857	6.61	0.011
C*RC	1	1.1020	1.2024	1.20235	34.75	0.000
Special Cubic	6	1.0575	1.0575	0.17625	5.09	0.000
PR*C*P	1	0.2640	0.2748	0.27476	7.94	0.005
PR*WC*RC	1	0.1093	0.1013	0.10133	2.93	0.088
PR*WC*SC	1	0.1383	0.1227	0.12267	3.55	0.061
PR*RC*SC	1	0.1576	0.1661	0.16609	4.80	0.029
P*WC*RC	1	0.2230	0.1983	0.19831	5.73	0.017
P*WC*SC	1	0.1654	0.1654	0.16539	4.78	0.030
Residual Error	256	8.8565	8.8565	0.03460		
Total	275	17.8548				

Appendix C Cubic model analysis output for weed biomass (Bullock et al.)

The estimated coefficient value of the cubic model for herbage biomass yield (component proportion)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0216	0.0114	1.89	0.059	1.50
2	0.0087	0.0114	0.77	0.443	1.50
3	-0.0269	0.0114	-2.36	0.019	1.50
PR	0.2294	0.0473	*	*	3.44
C	0.3322	0.0468	*	*	3.37
P	0.7572	0.0518	*	*	4.13
WC	0.7361	0.0474	*	*	3.47
RC	0.6367	0.0474	*	*	3.47
SC	1.1297	0.0519	*	*	4.16
PR*P	-0.984	0.197	-4.99	0.000	1.92
PR*WC	-1.597	0.226	-7.06	0.000	2.53
PR*RC	-1.093	0.210	-5.21	0.000	2.18
PR*SC	-1.953	0.214	-9.13	0.000	2.26
C*P	-0.863	0.197	-4.37	0.000	1.92
C*WC	-1.364	0.197	-6.92	0.000	1.92
C*RC	-0.831	0.197	-4.22	0.000	1.92
C*SC	-1.834	0.197	-9.30	0.000	1.92
P*WC	-1.691	0.211	-8.03	0.000	2.19
P*RC	-1.124	0.227	-4.96	0.000	2.54
P*SC	-1.339	0.214	-6.25	0.000	2.27
WC*SC	-1.226	0.227	-5.41	0.000	2.54
RC*SC	-0.656	0.227	-2.90	0.004	2.54
PR*WC*RC	-4.70	1.44	-3.27	0.001	1.64
PR*WC*SC	-2.78	1.53	-1.81	0.071	1.87
P*WC*RC	-3.38	1.44	-2.36	0.019	1.64
P*RC*SC	-3.25	1.53	-2.12	0.035	1.87
WC*RC*SC	-4.25	1.44	-2.96	0.003	1.64

Cubic model summary for weed biomass (Bullock et al.)

S	R-sq	R-sq[adj]	PRESS	R-sq(pred)
0.109183	79.15%	76.98%	3.70277	74.00%

Analysis of Variance for weed biomass (Bullock et al.)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.0880	0.0880	0.02935	2.46	0.063
Regression	23	11.1825	11.1825	0.48620	40.79	0.000
Linear	5	4.3205	2.9183	0.58367	48.96	0.000
Quadratic	13	6.3970	4.5289	0.34838	29.22	0.000
PR*P	1	0.0631	0.2969	0.29687	24.90	0.000
PR*WC	1	0.7933	0.5942	0.59416	49.84	0.000
PR*RC	1	0.3935	0.3231	0.32307	27.10	0.000
PR*SC	1	1.0703	0.9943	0.99426	83.40	0.000
C*P	1	0.0466	0.2280	0.22805	19.13	0.000
C*WC	1	0.3143	0.5712	0.57124	47.92	0.000
C*RC	1	0.1078	0.2120	0.21202	17.79	0.000
C*SC	1	0.8221	1.0301	1.03012	86.41	0.000
P*WC	1	0.7394	0.7688	0.76875	64.49	0.000
P*RC	1	0.5201	0.2929	0.29294	24.57	0.000
P*SC	1	0.5491	0.4654	0.46536	39.04	0.000
WC*SC	1	0.6705	0.3488	0.34883	29.26	0.000
RC*SC	1	0.3070	0.1000	0.09995	8.38	0.004
Special Cubic	5	0.4651	0.4651	0.09302	7.80	0.000
PR*WC*RC	1	0.1884	0.1276	0.12764	10.71	0.001
PR*WC*SC	1	0.0373	0.0392	0.03919	3.29	0.071
P*WC*RC	1	0.0845	0.0662	0.06619	5.55	0.019
P*RC*SC	1	0.0504	0.0535	0.05353	4.49	0.035
WC*RC*SC	1	0.1045	0.1045	0.10451	8.77	0.003
Residual Error	249	2.9683	2.9683	0.01192		
Total	275	14.2389				

Appendix D Estimated regression coefficient values (quadratic model) for ME content of herbage biomass

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	-0.0463	0.0181	-2.56	0.013	1.50
2	0.0348	0.0181	1.92	0.059	1.50
3	0.0128	0.0181	0.71	0.482	1.50
PR	11.2439	0.0487	*	*	2.26
C	10.6658	0.0487	*	*	2.26
P	10.8660	0.0487	*	*	2.26
WC	10.7146	0.0487	*	*	2.26
RC	10.5367	0.0487	*	*	2.26
SC	10.7783	0.0487	*	*	2.26
PR*C	0.162	0.237	0.69	0.496	1.48
PR*P	-0.216	0.237	-0.91	0.365	1.48
PR*WC	0.465	0.237	1.97	0.054	1.48
PR*RC	-0.315	0.237	-1.33	0.188	1.48
PR*SC	0.650	0.237	2.75	0.008	1.48
C*P	0.103	0.237	0.44	0.663	1.48
C*WC	0.341	0.237	1.44	0.154	1.48
C*RC	0.123	0.237	0.52	0.605	1.48
C*SC	0.273	0.237	1.15	0.253	1.48
P*WC	0.325	0.237	1.38	0.174	1.48
P*RC	-0.025	0.237	-0.11	0.916	1.48
P*SC	-0.097	0.237	-0.41	0.683	1.48
WC*RC	0.314	0.237	1.33	0.189	1.48
WC*SC	0.982	0.237	4.15	0.000	1.48
RC*SC	0.004	0.237	0.02	0.986	1.48

Quadratic model summary for ME

S	R-sq	R-sq[adj]	PRESS	R-sq[pred]
0.0978769	82.14%	75.72%	1.17231	65.85%

Analysis of variance for ME (component proportion)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.07744	0.07744	0.025814	2.69	0.053
Regression	20	2.74275	2.74275	0.137137	14.32	0.000
Linear	5	2.43281	1.18598	0.237195	24.76	0.000
Quadratic	15	0.30994	0.30994	0.020663	2.16	0.018
PR*C	1	0.00137	0.00450	0.004501	0.47	0.496
PR*P	1	0.01170	0.00798	0.007978	0.83	0.365
PR*WC	1	0.00989	0.03701	0.037009	3.86	0.054
PR*RC	1	0.02797	0.01696	0.016962	1.77	0.188
PR*SC	1	0.05821	0.07222	0.072219	7.54	0.008
C*P	1	0.00129	0.00183	0.001831	0.19	0.663
C*WC	1	0.00169	0.01993	0.019931	2.08	0.154
C*RC	1	0.00237	0.00259	0.002594	0.27	0.605
C*SC	1	0.00676	0.01275	0.012753	1.33	0.253
P*WC	1	0.00588	0.01813	0.018128	1.89	0.174
P*RC	1	0.00013	0.00011	0.000107	0.01	0.916
P*SC	1	0.00780	0.00162	0.001616	0.17	0.683
WC*RC	1	0.00640	0.01689	0.016892	1.76	0.189
WC*SC	1	0.16848	0.16517	0.165173	17.24	0.000
RC*SC	1	0.00000	0.00000	0.000003	0.00	0.986
Residual Error	64	0.61311	0.61311	0.009580		
Total	87	3.43330				

Appendix E Estimated regression coefficient values of herbage CP (%) content (component proportion)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	-0.171	0.126	-1.36	0.178	1.50
2	-0.281	0.126	-2.24	0.029	1.50
3	0.322	0.126	2.56	0.013	1.50
PR	16.851	0.338	*	*	2.26
C	18.627	0.338	*	*	2.26
P	21.648	0.338	*	*	2.26
WC	23.733	0.338	*	*	2.26
RC	24.310	0.338	*	*	2.26
SC	20.726	0.338	*	*	2.26
PR*C	-5.70	1.64	-3.47	0.001	1.48
PR*P	-5.91	1.64	-3.60	0.001	1.48
PR*WC	-5.96	1.64	-3.62	0.001	1.48
PR*RC	0.56	1.64	0.34	0.735	1.48
PR*SC	-8.17	1.64	-4.97	0.000	1.48
C*P	-4.14	1.64	-2.52	0.014	1.48
C*WC	-3.89	1.64	-2.37	0.021	1.48
C*RC	2.26	1.64	1.38	0.174	1.48
C*SC	-2.35	1.64	-1.43	0.158	1.48
P*WC	-5.36	1.64	-3.26	0.002	1.48
P*RC	-0.99	1.64	-0.60	0.550	1.48
P*SC	1.61	1.64	0.98	0.332	1.48
WC*RC	1.58	1.64	0.96	0.339	1.48
WC*SC	11.01	1.64	6.70	0.000	1.48
RC*SC	9.20	1.64	5.60	0.000	1.48

Quadratic model summary for CP content

S	R-sq	R-sq[adj]	PRESS	R-sq (pred)
0.680027	95.36%	93.69%	57.0670	91.05%

Analysis of variance for Crude protein (component proportion)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	5.027	5.027	1.6756	3.62	0.018
Regression	20	603.206	603.206	30.1603	65.22	0.000
Linear	5	529.042	167.076	33.4153	72.26	0.000
Quadratic	15	74.165	74.165	4.9443	10.69	0.000
PR*C	1	0.684	5.563	5.5633	12.03	0.001
PR*P	1	0.947	5.986	5.9861	12.94	0.001
PR*WC	1	3.967	6.074	6.0742	13.14	0.001
PR*RC	1	0.134	0.053	0.0533	0.12	0.735
PR*SC	1	20.819	11.416	11.4157	24.69	0.000
C*P	1	0.500	2.927	2.9270	6.33	0.014
C*WC	1	2.748	2.589	2.5889	5.60	0.021
C*RC	1	0.904	0.876	0.8757	1.89	0.174
C*SC	1	4.916	0.942	0.9421	2.04	0.158
P*WC	1	6.901	4.917	4.9172	10.63	0.002
P*RC	1	0.449	0.167	0.1669	0.36	0.550
P*SC	1	0.179	0.443	0.4428	0.96	0.332
WC*RC	1	0.165	0.429	0.4285	0.93	0.339
WC*SC	1	16.373	20.730	20.7302	44.83	0.000
RC*SC	1	14.478	14.478	14.4781	31.31	0.000
Residual Error	64	29.596	29.596	0.4624		
Total	87	637.829				

Appendix F Functional component analysis

Estimated Regression Coefficients for DMY (Quadratic model)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.1258	0.0221	5.69	0.000	1.50
2	0.0200	0.0221	0.90	0.366	1.50
3	-0.1403	0.0221	-6.34	0.000	1.50
G	1.4296	0.0564	*	*	3.37
AL	1.3195	0.0999	*	*	4.06
PeL	1.5792	0.0564	*	*	3.37
P	1.4873	0.0999	*	*	4.06
G*AL	0.842	0.280	3.00	0.003	2.52
G*PeL	1.339	0.201	6.67	0.000	3.18
G*P	0.753	0.280	2.69	0.008	2.52
AL*PeL	0.542	0.280	1.93	0.054	2.52
AL*P	0.281	0.384	0.73	0.465	1.93
PeL*P	0.315	0.280	1.12	0.263	2.52

Model summary

S	R-sq	R-sq[adj]	PRESS	R-sq(pred)
0.212256	33.64%	30.61%	12.8974	27.77%

Analysis of Variance for DMY (Functional component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	2.4807	2.4807	0.82691	18.35	0.000
Regression	9	3.5253	3.5253	0.39170	8.69	0.000
Linear	3	0.9387	0.2925	0.09752	2.16	0.093
Quadratic	6	2.5866	2.5866	0.43109	9.57	0.000
G*AL	1	0.2298	0.4063	0.40628	9.02	0.003
G*PeL	1	1.8308	2.0051	2.00512	44.51	0.000
G*P	1	0.3005	0.3253	0.32535	7.22	0.008
AL*PeL	1	0.1514	0.1686	0.16862	3.74	0.054
AL*P	1	0.0173	0.0241	0.02412	0.54	0.465
PeL*P	1	0.0568	0.0568	0.05675	1.26	0.263
Residual Error	263	11.8488	11.8488	0.04505		
Lack-of-Fit	131	5.7285	5.7285	0.04373	0.94	0.631
Pure Error	132	6.1203	6.1203	0.04637		
Total	275	17.8548				

Quadratic model analysis for weed biomass (DMY-W)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0216	0.0130	1.66	0.099	1.50
2	0.0087	0.0130	0.67	0.503	1.50
3	-0.0269	0.0130	-2.07	0.040	1.50
G	0.2922	0.0332	*	*	3.37
AL	1.1618	0.0588	*	*	4.06
PeL	0.6577	0.0332	*	*	3.37
P	0.7782	0.0588	*	*	4.06
G*AL	-1.969	0.165	-11.94	0.000	2.52
G*PeL	-1.325	0.118	-11.22	0.000	3.18
G*P	-0.927	0.165	-5.62	0.000	2.52
AL*PeL	-1.273	0.165	-7.72	0.000	2.52
AL*P	-1.516	0.226	-6.70	0.000	1.93
PeL*P	-1.617	0.165	-9.80	0.000	2.52

Model summary for weed biomass (DMY-W)

S	R-sq	R-sq[adj]	PRESS	R-sq[pred]
0.124882	71.19%	69.88%	4.55473	68.01%

Analysis of Variance for Weed Biomass (DMY-W) (Functional component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.0880	0.0880	0.02935	1.88	0.133
Regression	9	10.0492	10.0492	1.11658	71.60	0.000
Linear	3	3.9079	2.9517	0.98390	63.09	0.000
Quadratic	6	6.1412	6.1412	1.02354	65.63	0.000
G*AL	1	1.5744	2.2228	2.22277	142.53	0.000
G*PeL	1	1.5954	1.9636	1.96357	125.91	0.000
G*P	1	0.2526	0.4921	0.49209	31.55	0.000
AL*PeL	1	0.7099	0.9288	0.92878	59.55	0.000
AL*P	1	0.5097	0.7001	0.70013	44.89	0.000
PeL*P	1	1.4992	1.4992	1.49922	96.13	0.000
Residual Error	263	4.1016	4.1016	0.01560		
Lack-of-Fit	131	2.6777	2.6777	0.02044	1.89	0.000
Pure Error	132	1.4239	1.4239	0.01079		
Total	275	14.2389				

Estimated Regression Coefficients for DMY W (component proportions)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0216	0.0128	1.69	0.093	1.50
2	0.0087	0.0128	0.68	0.495	1.50
3	-0.0269	0.0128	-2.10	0.036	1.50
G	0.2733	0.0335	*	*	3.56
AL	1.1289	0.0594	*	*	4.31
PeL	0.6298	0.0335	*	*	3.56
P	0.7423	0.0594	*	*	4.31
G*AL	-1.786	0.199	-8.98	0.000	3.79
G*PeL	-1.125	0.134	-8.43	0.000	4.22
G*P	-0.716	0.199	-3.60	0.000	3.79
AL*PeL	-0.942	0.199	-4.74	0.000	3.79
AL*P	-1.229	0.293	-4.19	0.000	3.38
PeL*P	-1.259	0.199	-6.33	0.000	3.79
G*AL*PeL	-1.769	0.880	-2.01	0.045	2.75
G*AL*P	-0.05	1.23	-0.04	0.968	2.27
G*PeL*P	-2.022	0.880	-2.30	0.022	2.75
AL*PeL*P	-2.75	1.23	-2.23	0.027	2.27

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.122652	72.64%	70.95%	4.48454	68.50%

Analysis of Variance for DMY W (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.0880	0.0880	0.02935	1.95	0.122
Regression	13	10.2545	10.2545	0.78881	52.44	0.000
Linear	3	3.9079	2.7665	0.92216	61.30	0.000
Quadratic	6	6.1412	2.3010	0.38349	25.49	0.000
G*AL	1	1.5744	1.2141	1.21408	80.70	0.000
G*PeL	1	1.5954	1.0682	1.06815	71.00	0.000
G*P	1	0.2526	0.1953	0.19534	12.98	0.000
AL*PeL	1	0.7099	0.3374	0.33737	22.43	0.000
AL*P	1	0.5097	0.2637	0.26366	17.53	0.000
PeL*P	1	1.4992	0.6032	0.60317	40.10	0.000
Special Cubic	4	0.2054	0.2054	0.05134	3.41	0.010
G*AL*PeL	1	0.0551	0.0609	0.06086	4.05	0.045
G*AL*P	1	0.0002	0.0000	0.00002	0.00	0.968
G*PeL*P	1	0.0751	0.0795	0.07947	5.28	0.022
AL*PeL*P	1	0.0749	0.0749	0.07485	4.98	0.027
Residual Error	259	3.8963	3.8963	0.01504		
Lack-of-Fit	127	2.4724	2.4724	0.01947	1.80	0.000
Pure Error	132	1.4239	1.4239	0.01079		
Total	275	14.2389				

Appendix G Estimated Regression Coefficients for RGRD (component proportions)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.057	0.251	0.23	0.822	1.50
2	0.045	0.251	0.18	0.858	1.50
3	-0.238	0.251	-0.95	0.348	1.50
PR	-1.90	1.48	*	*	8.75
C	-0.22	1.48	*	*	8.75
P	0.11	1.48	*	*	8.75
WC	-2.194	0.971	*	*	3.75
RC	1.423	0.971	*	*	3.75
SC	2.909	0.971	*	*	3.75
PR*C	-0.15	5.49	-0.03	0.979	6.00
PR*P	7.14	5.49	1.30	0.201	6.00
PR*WC	3.69	4.48	0.82	0.416	4.00
PR*RC	-1.44	4.48	-0.32	0.749	4.00
C*P	7.04	5.49	1.28	0.207	6.00
C*WC	5.39	4.48	1.20	0.236	4.00
C*RC	-2.55	4.48	-0.57	0.573	4.00
P*WC	-1.76	4.48	-0.39	0.696	4.00
P*RC	-8.85	4.48	-1.97	0.055	4.00

Model Summary

S	R-sq	R-sq[adj]	PRESS	R-sq(pred)
1.12123	60.31%	44.24%	107.757	18.99%

Analysis of Variance for RGRD (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	1.206	1.2060	0.40198	0.32	0.811
Regression	14	79.011	79.0110	5.64364	4.49	0.000
Linear	5	44.584	17.0893	3.41786	2.72	0.032
Quadratic	9	34.427	34.4268	3.82521	3.04	0.007
PR*C	1	6.802	0.0009	0.00089	0.00	0.979
PR*P	1	6.357	2.1252	2.12522	1.69	0.201
PR*WC	1	0.210	0.8489	0.84889	0.68	0.416
PR*RC	1	0.521	0.1302	0.13018	0.10	0.749
C*P	1	11.588	2.0648	2.06475	1.64	0.207
C*WC	1	3.162	1.8188	1.81882	1.45	0.236
C*RC	1	0.295	0.4049	0.40491	0.32	0.573
P*WC	1	0.592	0.1941	0.19413	0.15	0.696
P*RC	1	4.899	4.8993	4.89935	3.90	0.055
Residual Error	42	52.801	52.8007	1.25716		
Total	59	133.018				

Appendix H The effect of sown proportion on annual average biomass yield of pasture swards (Quadratic model analysis).

Quadratic model analysis for biomass yield of 2018/19 production year

Y = 1.83 PR + 1.31 Co + 1.78 Pl + 1.54 WC + 1.74 RC + 1.22 SC + 0.01 PRC - 0.11 PRPl + 0.04 PRWC + 1.68 **PRRC** + 2.25 **PRSC** + 0.53 COPl + 0.91 **COWC** + 2.13 **CORC** + 1.15 **COSC** + 0.49 PlWC + 0.1 PlRC + 0.90 **PlSC** + 1.32 **WCRC** + 0.58 WCSC + 1.08 **RCSC**.

(R^2 = 42.16 %, R^2[adj] = 36.88 %, p=0.000).Quadratic model analysis for biomass yield of 2019/20 production year

Y = 1.59 PR + 1.30 CO + 1.37 Pl + 1.24 WC + 1.58 RC + 1.34 SC + 0.41 PRC + 0.91 **PRPl** + 0.47 **PRWC** + 0.64 **PRRC** + 0.65 PRSC + 0.64 COPl + 0.70 COWC + 2.10 **CORC** + 0.73 COSC + 0.79 PlWC + 0.02 PlRC + 0.17 PlSC + 0.90 **WCRC** + 0.55 WCSC + 0.19 RCSC.

(R^2 = 31.53 %, R^2(adj) = 25.28 %, p=0.000).

C3.3 Quadratic model analysis for biomass yield of 2020/21 production year

Y = 1.18 PR + 0.96 CO + 1.38 Pl + 1.32 WC + 1.59 RC + 1.41 SC + 1.71 **PRC** + 1.57 **PRPl** + 2.60 **PRWC** + 2.60 **PRRC** + 1.36 **PRSC** + 1.09 COPl + 1.19 **COWC** + 1.90 **CORC** – 0.63 COSC + 0.34 PlWC + 0.49 PlRC - 0.22 PlSC + 0.22 WCRC + 0.82 WCSC + 0.36 RCSC.

(R^2 = 51.57 %, R^2(adj) = 47.15 %, p=0.000).

Appendix I Interaction coefficient value for above-ground biomass yield (TY) from the quadratic model analysis at Lincoln University, Canterbury, New Zealand (2018 to 2021).

Date of sampling	PR.C	PR.P	PR.WC	PR.RC	PR.SC	C.P	C.WC	C.RC	C.SC	P.WC	P.RC	P.SC	WC.RC	WC.SC	RC.SC
14/09/2018	0.68	0.56	-1.08	0.78	**1.93**	0.34	-0.23	1.48	0.25	0.83	-1.36	-1.35	0.51	-0.42	**-1.36**
19/10/2018	-0.18	0.64	0.51	0.42	**1.89**	0.73	0.44	0.63	**1.64**	-0.06	-0.79	0.38	**0.71**	0.82	0.32
29/11/2018	-0.63	0.10	0.14	**2.35**	**3.09**	-0.93	**1.36**	1.00	0.19	-1.26	0.11	0.60	0.70	**1.49**	0.90
08/01/2019	-2.05	-1.63	0.70	1.54	0.64	1.05	1.89	**3.88**	0.26	-0.58	0.28	0.66	**3.67**	-1.76	2.15
24/02/2019	0.66	-0.44	-0.65	1.66	-0.21	1.41	1.11	**4.26**	-1.23	1.18	0.25	**1.96**	1.15	-1.13	**2.32**
28/03/2019	-0.78	-0.98	0.25	**2.56**	**3.71**	0.72	0.52	**2.09**	**4.40**	**0.69**	0.05	**1.98**	1.17	**3.19**	**2.90**
22/05/2019	**2.42**	0.94	**2.94**	**2.49**	**4.72**	**0.41**	1.34	**1.59**	**2.56**	**2.67**	**1.55**	**2.08**	**1.30**	**1.92**	0.39
30/07/2019	**1.86**	-0.19	0.75	0.46	-1.26	0.92	0.00	0.68	-0.59	0.71	0.01	-1.49	-0.20	-1.29	-1.28
13/09/2019	-0.45	**1.34**	-1.19	-2.25	-0.39	-0.54	-1.19	0.26	-1.32	0.31	-0.66	-0.18	0.51	0.36	-0.32
21/10/2019	-0.35	1.16	-0.29	0.31	0.93	-0.17	0.44	**1.93**	0.33	0.77	-0.44	-1.06	0.43	0.00	-0.55
27/11/2019	0.98	1.47	0.41	-0.10	0.21	0.92	1.05	**2.69**	**2.01**	0.62	-0.09	0.75	-0.04	1.64	0.68
09/01/2019	-0.37	0.19	-1.74	1.43	-1.13	1.03	0.96	**1.90**	0.25	0.62	1.43	-1.11	**2.76**	-0.14	0.34
18/02/2020	1.36	1.88	0.81	**2.55**	-1.66	1.38	-0.04	**3.31**	0.95	**3.21**	**2.34**	0.61	1.47	-0.06	-0.48
27/03/2020	0.59	1.72	**2.56**	**2.7**	**3.93**	0.10	**2.48**	**4.61**	**2.50**	-0.82	-1.43	1.16	1.20	**2.53**	1.38
28/05/2020	-0.34	-0.24	**2.49**	0.04	**4.60**	1.53	1.92	1.47	1.77	0.88	-0.99	**2.71**	1.09	1.38	1.81
28/07/2020	1.22	-0.51	0.91	0.90	0.80	0.08	-1.64	0.74	0.73	-0.23	-0.49	-0.10	-0.57	0.31	0.20
14/09/2020	**1.83**	1.11	0.53	0.45	0.72	0.72	0.10	1.50	-2.32	1.26	1.40	0.84	-0.33	0.24	0.34
21/10/2020	**2.57**	0.24	0.44	-0.80	0.03	1.62	-0.28	1.22	-0.70	-0.64	-0.61	-0.61	0.73	-1.75	-1.09
30/11/2020	1.81	**3.39**	1.82	**5.44**	**3.14**	1.02	**3.37**	1.86	**2.95**	-0.11	2.01	0.37	-0.45	2.32	1.69
12/01/2021	**2.84**	**3.22**	**3.35**	**7.46**	-0.09	**2.94**	**2.78**	4.74	-3.85	0.32	1.40	-0.97	1.64	2.31	2.14
17/02/2021	**2.49**	**3.73**	**6.02**	2.42	**3.04**	1.09	1.43	**1.92**	-1.45	1.41	0.43	-0.58	0.36	0.61	-0.36
25/03/2021	1.04	1.43	**4.91**	**3.19**	**1.76**	0.58	1.56	**2.49**	0.52	1.19	0.85	0.66	0.59	**1.69**	0.25
25/05/2021	-0.13	-0.05	**2.82**	**1.75**	1.45	0.66	2.15	0.88	-0.27	-0.47	-1.06	-1.38	-0.17	0.88	-0.31

Appendix J Interaction coefficient value for Weed biomass yield (DM-W) from the quadratic model analysis at Lincoln University, Canterbury, New Zealand

Date of sampling	PR.C	PR.P	PR.WC	PR.RC	PR.SC	C.P	C.WC	C.RC	C.SC	P.WC	P.RC	P.SC	WC.RC	WC.SC	RC.SC
14/09/2018	-0.23	-0.58	**-1.49**	-0.07	0.34	-0.09	-0.48	0.42	-0.06	0.16	**-1.13**	-0.95	0.05	-0.80	-0.58
19/10/2018	**-1.02**	-0.46	**-1.33**	**-0.77**	**-0.63**	-0.9	0.15	0.29	**0.50**	-0.21	**-0.53**	**0.13**	0.40	-0.18	0.09
29/11/2018	**-1.49**	**-1.60**	**-2.62**	**-1.81**	**-1.43**	**-1.38**	-0.75	-0.90	**-1.08**	**-2.03**	-0.64	-1.32	-0.73	-0.46	0.77
08/01/2019	-1.33	**-2.18**	**-4.76**	**-2.70**	**-5.03**	**-2.23**	**-4.13**	**-2.29**	**-2.82**	**-3.89**	-1.16	**-3.87**	**-1.75**	**-3.52**	**-1.93**
24/02/2019	-0.04	**-0.76**	**-1.41**	**-0.70**	**-2.44**	-0.22	**-1.13**	0.21	**-2.03**	**-1.62**	**-0.87**	**-2.46**	-0.26	**-0.29**	**-0.98**
28/03/2019	**-0.92**	**-0.70**	**-1.75**	**-0.90**	-0.16	**-0.71**	**-1.15**	-0.47	0.64	**-1.60**	**-0.56**	**-0.73**	014	-0.36	0.38
22/05/2019	**-0.37**	-0.34	**-1.23**	**-1.29**	**-1.42**	-0.35	**-0.63**	-0.08	**-0.79**	**-1.11**	**-0.93**	**-0.74**	-0.18	**-0.73**	**-0.47**
30/07/2019	-0.05	-0.32	**-0.90**	**-1.24**	**-2.94**	0.10	**-0.72**	**-0.46**	**-2.34**	**-0.73**	**-1.01**	**-2.12**	**-0.48**	**-1.77**	**-1.84**
13/09/2019	**-0.49**	-0.44	**-1.49**	**-2.12**	**-2.08**	-0.44	**-1.53**	**-0.96**	**-2.68**	**-0.70**	**-1.30**	**-1.75**	**-0.64**	**-1.98**	**-1.14**
21/10/2019	-0.42	**-1.54**	**-2.66**	**-2.86**	**-3.90**	**-1.35**	**-2.52**	**-1.62**	**-3.09**	**-2.84**	**-1.60**	**-1.83**	-0.79	**-2.94**	**-2.63**
27/11/2019	0.25	-0.91	**-3.02**	**-2.58**	**-2.49**	-0.36	**-2.41**	-0.74	**-1.46**	**-1.82**	**-1.79**	-0.48	**-1.99**	**-2.10**	**1.30**
09/01/2019	-0.33	**-0.82**	**-1.77**	**-2.42**	**-2.70**	-0.48	**-0.74**	**-0.69**	**-2.15**	-0.49	**-1.19**	**-2.04**	-0.52	**-2.34**	**-2.19**
18/02/2020	0.39	**-0.91**	**-1.62**	**-1.52**	**-2.81**	-0.25	**-1.28**	-0.65	**-3.55**	**-1.80**	**-1.46**	**-3.00**	-0.49	**-2.46**	**-2.94**
27/03/2020	-0.24	**-2.14**	**-1.61**	-0.33	-0.22	**-2.03**	**1.40**	**-1.72**	**-0.94**	**-1.87**	**-1.93**	-0.14	**-0.86**	-0.17	0.36
28/05/2020	0.28	**-0.60**	**-1.34**	**-1.05**	-0.48	**-0.68**	**-1.15**	**-1.10**	**-0.59**	**-0.60**	**-1.30**	**0.80**	-0.38	-0.15	0.26
28/07/2020	0.30	-0.41	**-1.69**	**-0.57**	**-0.57**	-0.25	**-1.65**	**-0.58**	-0.60	**-1.61**	**-0.90**	0.02	**-1.12**	0.42	**0.56**
14/09/2020	**0.88**	**-1.05**	**-2.57**	**-2.05**	**-3.38**	-0.77	**-2.18**	**-1.57**	**-3.34**	**-2.39**	-0.71	**-1.79**	**-2.48**	**-0.92**	**-1.55**
21/10/2020	**1.28**	-0.88	**-3.46**	**-2.54**	**-4.08**	-0.75	**-3.16**	**-1.78**	**-3.68**	**-3.18**	**-2.33**	**-2.53**	**-1.30**	**-3.62**	**-2.04**
30/11/2020	1.08	**-1.75**	**-4.555**	-1.14	**-3.08**	**-2.73**	**-4.04**	-1.09	**-2.19**	**-5.66**	**3.91**	**-1.73**	**-2.60**	**-3.01**	**-2.24**
12/01/2021	1.41	-1.25	**-3.06**	-0.75	-3.88	-0.81	-2.00	-1.02	**-4.62**	**-5.84**	**-4.69**	**-3.89**	-1.05	**-4.44**	-1.95
17/02/2021	0.38	0.37	-0.27	-0.87	**-0.93**	-0.59	-0.01	**-0.88**	**-1.34**	-0.52	**-1.17**	**-1.62**	-0.73	**-0.91**	**-1.01**
25/03/2021	0.47	-0.49	-0.41	-0.26	**-1.18**	**-0.99**	-0.82	-0.51	**-1.11**	**--0.72**	**-1.23**	**-0.61**	-0.56	**-0.84**	**-0.78**
25/05/2021	0.40	**-2.54**	**-1.07**	-0.32	**-2.59**	**-2.24**	**-1.13**	**-0.85**	**-2.58**	**-1.91**	**-1.76**	**-1.81**	-0.69	**-1.33**	**-1.25**

Appendix K Interaction coefficient value for ME (MJ ME/kg DM) content of binary mixtures obtained from the quadratic model at Lincoln University, Canterbury (2018 to 2021)

Date of sampling	PR.C	PR.P	PR.WC	PR.RC	PR.SC	C.P	C.WC	C.RC	C.SC	P.WC	P.RC	P.SC	WC.RC	WC.SC	RC.SC
29/11/2018	-0.17	-0.06	**-2.58**	-0.38	0.87	-0.37	-0.72	-0.35	0.55	0.44	-0.35	-0.01	-0.32	-0.12	0.74
08/01/2019	**1.83**	1.16	-1.11	**-1.7**	**5.19**	-1.09	0.10	-0.29	**2.70**	-1.13	0.39	-1.47	**1.73**	**2.54**	-1.05
24/02/2019	0.94	-0.48	0.48	-1.71	**1.99**	-0.70	0.93	**-1.90**	0.63	-1.04	-0.59	-1.59	**-1.77**	**2.69**	0.22
28/03/2019	0.08	-0.50	-0.81	-0.67	3.17	-0.74	-0.12	**-1.39**	**4.16**	**-1.52**	-0.41	2.24	-0.48	**5.55**	3.72
25/05/2019	0.45	-1.15	-0.67	0.08	**3.79**	0.02	1.24	0.31	**3.05**	0.50	0.61	**2.83**	-0.40	**4.48**	**4.02**
30/07/2019	-0.93	0.97	0.77	-1.51	**-2.46**	1.06	0.18	1.83	-1.80	0.73	0.69	-0.61	0.38	0.15	-0.99
13/09/2019	0.28	0.01	0.53	0.26	-0.56	**1.39**	1.24	0.81	-0.57	-0.24	0.49	-0.70	0.48	-0.14	0.27
21/10/2019	-0.81	-0.08	-1.02	-0.72	-0.89	0.73	-0.86	-0.50	0.46	0.30	0.47	0.68	0.10	0.08	0.52
27/11/2019	**2.47**	-0.96	**2.92**	-0.60	1.82	-0.52	0.71	0.35	0.89	**2.22**	-1.27	1.96	3.37	**5.71**	0.90
09/01/2020	1.41	0.51	**1.64**	0.63	1.34	-0.42	-0.26	0.06	-0.82	-1.09	0.57	-0.66	1.12	0.75	1.19
18/02/2020	0.34	1.09	**2.00**	-0.50	2.16	0.07	0.05	-1.06	1.36	-0.73	-0.71	0.21	-0.18	0.05	0.45
27/03/2020	-0.64	0.05	1.31	1.23	0.22	1.21	0.57	-2.14	0.21	**3.36**	0.82	1.61	2.33	1.11	-2.45
28/05/2020	0.08	-0.43	1.88	-0.11	3.72	0.54	1.66	3.24	2.74	-0.89	1.17	-0.11	1.60	2.13	0.37
28/07/2020	-2.69	2.25	-2.98	2.49	-2.26	0.24	0.37	5.26	-3.09	2.86	2.72	-1.24	2.70	-1.05	-0.64
14/09/2020	-2.01	**-3.23**	-0.94	-0.77	-1.36	-0.03	-1.05	-0.33	-1.38	-1.26	-066	-1.63	-0.46	-0.86	-1.36
21/10/2020	**0.01**	-0.85	0.17	-1.44	-0.86	0.12	-0.77	-1.61	-0.35	-0.19	-0.89	0.54	-0.99	0.95	-1.10
30/11/2020	0.33	-0.13	1.19	-1.02	0.30	-0.50	-1.66	1.93	-1.47	0.81	-0.31	1.23	-0.39	0.72	0.16
12/01/2021	-0.31	-1.00	1.61	0.04	0.40	-0.46	1.25	-0.78	0.27	1.89	0.22	1.66	0.43	0.91	**-0.01**
17/02/2021	1.13	-0.91	0.55	-3.25	1.74	1.47	2.87	0.11	2.72	1.18	0.20	0.98	-0.75	1.49	-0.37
25/03/2021	-033	-1.28	**2.55**	-0.16	-0.17	-0.54	-0.01	**-2.79**	-0.64	-0.63	**-2.47**	-2.12	-1.77	0.80	0.32
25/05/2021	1.69	0.26	2.03	2.20	1.56	0.46	1.21	1.61	2.17	1.00	-1.46	0.72	-0.37	-0.61	1.20

Appendix L Interaction coefficient value for CP (%) content of binary mixtures obtained from quadratic model analysis of multi-species experiment at Lincoln University, Canterbury (2018 to 2021).

Date of sampling	PR.C	PR.P	PR.WC	PR.RC	PR.SC	C.P	C.WC	C.RC	C.SC	P.WC	P.RC	P.SC	WC.RC	WC.SC	RC.SC
29/11/2018	**-18.91**	**-15.22**	**-10.91**	**-12.03**	**-7.56**	**-10.63**	**-8.72**	5.75	3.60	**-8.83**	-4.34	-0.84	-3.50	**-0.39**	-4.02
08/01/2019	**8.88**	-4.16	1.83	**-9.91**	**-18.17**	**-9.13**	1.67	**-11.24**	**10.54**	**-9.81**	-3.37	-2.06	4.64	**9.76**	3.02
24/02/2019	5.31	1.07	2.34	5.25	-6.47	-7.17	7.54	-1.83	4.47	-9.74	-1.03	**-18.97**	-2.94	9.13	0.63
28/03/2019	-0.25	-3.59	-7.78	-3.41	**-41.7**	**-9.68**	-8.86	-7.09	-29.8	**-15.4**	-5.38	-35.9	-2.36	-19.8	-26.5
22/05/2019	-8.37	**-14.4**	**-24.92**	**-13.2**	**-19.4**	**-16.96**	-6.08	-2.61	**-8.90**	**-22.3**	**-14.67**	**-15.45**	0.07	-0.42	**-8.37**
30/07/2019	**-11.05**	0.30	-4.61	1.24	6.93	-1.00	-1.46	**8.47**	**-12.09**	-6.62	-0.43	-7.41	2.86	7.88	3.08
13/09/2019	**-9.92**	-4.17	**-21.57**	-8.50	-8.75	8.15	-5.49	-0.80	0.92	-7.60	2.23	0.25	0.38	7.09	4.84
21/10/2019	**-16.57**	**-14.37**	**-23**	-8.03	-1.29	-9.02	**-13.05**	**-1.3**	-7.26	-0.58	-1.31	3.3	1.96	**14.74**	**20.16**
27/11/2019	-7.48	-11.25	-8.99	-8.27	-11.36	-7.78	-7.78	7.45	**17.92**	3.03	-1.12	4.44	4.44	**32.77**	**20.88**
09/01/2019	-4.00	-6.21	-5.73	10.85	2.43	-3.68	11.32	0.51	-3.84	-2.07	-0.82	11.59	2.86	2.15	**16.75**
18/02/2020	-6.00	-9.4	**-25.39**	3.67	-4.63	-1.77	-7.61	3.89	-3.41	-9.56	2.62	-6.95	7.29	10.56	10.61
27/03/2020	**-11.19**	**-12.02**	**-18.5**	10.66	8.6	1.84	**-16.39**	-5.13	4.6	**-11.14**	2.66	20.4	-1.37	34.3	31.1
28/05/2020	-3.81	**-16.48**	**-15.41**	-5.00	-11.3	-2.42	**-16.09**	0.68	-2.10	**-14.63**	0.43	2.20	0.97	13.8	14.7
28/07/2020	-4.47	**-12.57**	**-8.3**	2.59	-4.15	**-13.61**	6.94	1.90	-7.07	5.43	1.84	5.46	**11.01**	**19.56**	**17.6**
14/09/2020	-5.34	-4.95	-1.06	5.09	-7.20	3.42	0.10	-6.95	1.23	5.14	1.68	9.47	**14.4**	**13.07**	**17.86**
21/10/2020	10.91	-7.63	1.95	10.96	-8.10	-4.02	0.72	9.89	0.46	-13.57	9.57	14.02	8.75	**17.32**	11.94
30/11/2020	-0.46	1.85	10.62	**15.23**	-9.21	-3.90	-1.18	**12.99**	0.48	5.34	-1.80	**15.40**	-4.60	**12.34**	**15.87**
12/01/2021	4.89	11.72	18.95	**19.70**	3.97	3.54	6.87	13.52	-10.30	8.61	7.37	-2.42	3.15	15.48	**15.72**
17/02/2021	-9.11	6.09	11.51	14.59	**-17.73**	0.90	1.76	**16.87**	-5.07	-3.20	0.26	11.82	4.87	**16.74**	7.58
25/03/2021	-1.88	-1.21	**15.16**	**15.28**	-4.28	-6.96	-0.66	3.11	-9.87	-2.52	-8.29	-7.80	-5.78	10.44	9.86
25/05/2021	-9.17	7.69	10.76	**-13.19**	-11.73	5.37	-2.06	1.00	-7.14	-2.01	**14.98**	**20.34**	**-13.33**	-8.81	-3.60

Appendix M Mean Cu PAR energy of monocultures and binary swards (95 % confidence limits)

	14/09/2020	21/10/2020	30/11/2020	12/01/2021	17/02/2021	25/03/2021	25/05/2021
PR	139.3 (119.7,158.9)	123.2 (106.3,140.1)	69.2 (40.3,98.1)	26.1 (-3.0,55.2)	83.8 (43.6,124.0)	58.0 (28.0,88.1)	78.8 (49.6,108.0)
CF	93.2 (73.6,112.8)	138.8 (121.9,155.7)	106.8 (77.8,135.7)	65.6 (36.5,94.7)	168.1 (127.9,208.3)	111.0 (81.0,141.1)	141.2 (111.9,170.4)
PL	100.7 (81.1,120.3)	112.2 (95.3,129.2)	131.8 (102.8,160.7)	127.8 (98.7,156.9)	65.7 (25.5,105.9)	82.2 (52.2,112.2)	116.4 (87.2,145.6)
WC	104.0 (84.4,123.6)	122.7 (105.7,139.6)	110.3 (81.4,139.3)	109.9 (80.7,139.0)	30.8 (-9.4,71.0)	10.2 (-19.8,40.2)	55.7 (26.5,84.9)
RC	109.5 (89.9,129.1)	127.6 (110.7,144.5)	117.8 (88.9,146.8)	119.3 (90.2,148.4)	104.4 (64.2,144.6)	81.0 (51.0,111.0)	83.9 (54.7,113.1)
SC	88.8 (69.2,108.4)	110.1 (93.2,127.0)	121.9 (92.9,150.8)	82.9 (53.8,112.0)	124.0 (83.8,164.2)	85.7 (55.7,115.8)	137.4 (108.2,166.6)
PR CF	128.9 (109.3,148.5)	147.0 (130.1,164.0)	83.4 (54.5,112.4)	59.7 (30.6,88.8)	157.4 (117.2,197.7)	105.5 (75.4,135.5)	150.9 (121.6,180.1)
PR PL	101.4 (81.9,121.0)	135.2 (118.2,152.1)	130.2 (101.3,159.2)	101.1 (72.0,130.2)	190.1 (149.9,230.3)	106.5 (76.5,136.5)	156.6 (127.4,185.8)
PR WC	120.7 (101.1,140.3)	131.8 (114.9,148.7)	150.9 (121.9,179.8)	91.2 (62.1,120.3)	110.0 (69.7,150.2)	105.0 (75.0,135.0)	184.7 (155.5,213.9)
PR RC	116.0 (96.4,135.6)	169.4 (152.5,186.3)	107.6 (78.7,136.5)	86.8 (57.7,115.9)	140.7 (100.5,180.9)	119.0 (89.0,149.0)	126.5 (97.3,155.7)
PR SC	121.5 (101.9,141.1)	156.4 (139.5,173.3)	88.0 (59.0,116.9)	24.3 (-4.8,53.4)	139.7 (99.4,179.9)	85.6 (55.5,115.6)	152.3 (123.1,181.5)
CF PL	99.9 (80.4,119.5)	135.1 (118.2,152.1)	121.9 (93.0,150.8)	97.2 (68.1,126.3)	169.9 (129.7,210.1)	116.4 (86.4,146.5)	148.7 (119.4,177.9)
CF WC	89.2 (69.6,108.8)	136.9 (119.9,153.8)	139.1 (110.2,168.0)	83.5 (54.4,112.6)	135.6 (95.4,175.9)	92.9 (62.9,122.9)	146.8 (117.6,176.0)
CF RC	113.7 (94.1,133.3)	151.4 (134.5,168.3)	124.0 (95.1,152.9)	97.5 (68.4,126.6)	193.5 (153.3,233.7)	120.4 (90.4,150.4)	156.6 (127.4,185.8)
CF SC	88.0 (68.4,107.6)	136.8 (119.8,153.7)	130.1 (101.2,159.0)	75.5 (46.4,104.6)	188.8 (148.6,229.0)	136.6 (106.5,166.6)	156.2 (126.9,185.4)
WC PL	103.4 (83.8,122.9)	106.3 (89.4,123.2)	126.8 (97.9,155.8)	110.8 (81.7,139.9)	112.5 (72.3,152.7)	68.9 (38.9,99.0)	115.7 (86.5,144.9)
RC PL	127.3 (107.7,146.8)	106.0 (89.1,123.0)	108.0 (79.0,136.9)	136.9 (107.8,166.0)	138.5 (98.3,178.8)	91.7 (61.6,121.7)	109.2 (80.0,138.4)
SC PL	91.5 (71.9,111.1)	109.6 (92.6,126.5)	133.5 (104.5,162.4)	112.8 (83.7,141.9)	125.1 (84.9,165.3)	86.7 (56.7,116.8)	127.9 (98.7,157.2)
WC RC	105.1 (85.5,124.7)	117.1 (100.2,134.0)	120.5 (91.5,149.4)	117.0 (87.9,146.1)	116.9 (76.7,157.1)	80.3 (50.2,110.3)	110.9 (81.7,140.1)
WC SC	106.4 (86.8,126.0)	120.4 (103.5,137.3)	96.9 (68.0,125.8)	98.7 (69.6,127.8)	121.6 (81.4,161.8)	61.8 (31.8,91.8)	99.8 (70.6,129.1)
RC SC	104.9 (85.3,124.5)	104.7 (87.8,121.6)	106.7 (77.8,135.7)	115.7 (86.6,144.8)	118.6 (78.4,158.9)	73.7 (43.6,103.7)	103.0 (73.8,132.2)
Centroid	108.0 (88.4,127.6)	133.3 (116.4,150.2)	147.7 (118.7,176.6)	134.0 (104.9,163.1)	194.4 (154.2,234.6)	134.7 (104.7,164.7)	7.5 (129.3,187.7)

Appendix N RUE of functional group species

Functional groups	RUE Coefficient value	SE of coefficient value	R^2 (Adj)%	P value
Grass	1.25	0.02	99	0.01
Perennial clovers	1.69	0.04	98	0.01
Herbs	1.67	0.03	99	0.01

Appendix O RUE value of swards, biomass yield, weeds, and VWC%, and herbage N content in a pasture diversity experiment at Lincoln University, Canterbury, New Zealand, during 2020/21.

Sward type	RUE (g DM MJ^{-1})	Biomass yield (t/ha)	Weeds (%)	VWC (%)	Herbage N content (%)
PR	1.32	1.20	1	1	2.63
C	1.19	0.98	2	1	2.72
P	1.54	1.39	37	21	3.73
WC	1.62	1.37	47	0	3.60
RC	1.56	1.59	22	10	3.91
SC	1.77	1.50	49	9	3.14
PR*C	1.46	1.47	1	0	2.45
PR*P	1.28	1.68	2	10	3.07
PR*WC	1.55	1.89	3	0	3.26
PR*RC	1.68	1.98	2	4	3.58
PR*SC	1.46	1.54	0	1	2.55
C*P	1.24	1.44	2	10	3.09
C*WC	1.43	1.48	4	0	3.18
C*RC	1.35	1.85	3	3	3.53
C*SC	1.02	0.99	6	2	2.70
P*WC	1.51	1.40	28	0	3.63
P*RC	1.62	1.54	18	10	3.75
P*SC	1.12	1.18	26	34	3.72
WC*RC	1.57	1.56	29	0	3.80
WC*SC	1.53	1.43	41	0	3.81
RC*SC	1.79	1.52	27	12	3.95
Centroid	1.42	1.89	2	0	3.62
Mean	1.46	1.49	0.23	0.11	3.33
SE	0.10	0.15	0.07	0.04	0.11

Appendix P Diversity Interaction (DI) model output for canopy pre-grazing fraction interception (fPARi)

Linear model

Estimated coefficient value of fPARi (linear model) for monocultures

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	-0.0049	0.0138	-0.36	0.722	1.50
2	0.0141	0.0138	1.02	0.310	1.50
3	-0.0245	0.0138	-1.77	0.080	1.50
PR	0.8502	0.0177	*	*	1.02
C	0.8572	0.0206	*	*	1.01
P	0.8418	0.0212	*	*	1.03
WC	0.7760	0.0247	*	*	1.02
RC	0.8552	0.0235	*	*	1.03
SC	0.8006	0.0373	*	*	1.00

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.0748825	13.84%	5.12%	0.539933	0.00%

Analysis of Variance for fPARi (pre-grazing) (monocultures)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.02179	0.02333	0.007778	1.39	0.253
Regression	5	0.04938	0.04938	0.009875	1.76	0.131
Linear	5	0.04938	0.04938	0.009875	1.76	0.131
Residual Error	79	0.44298	0.44298	0.005607		
Total	87	0.51415				

Quadratic model

Estimated interaction coefficient values of pre-grazing fPARi for binary mixtures

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0013	0.0126	0.10	0.920	1.53
2	0.0167	0.0127	1.32	0.192	1.54
3	-0.0257	0.0128	-2.01	0.049	1.57
RG	0.7915	0.0236	*	*	2.20
C	0.8229	0.0297	*	*	2.58
P	0.8335	0.0290	*	*	2.33
WC	0.7515	0.0300	*	*	1.84
RC	0.8673	0.0334	*	*	2.54
SC	0.8063	0.0339	*	*	1.01
RG*C	0.372	0.281	1.32	0.190	1.27
RG*P	0.156	0.151	1.04	0.304	1.25
RG*WC	1.076	0.313	3.44	0.001	1.28
RG*RC	0.437	0.166	2.64	0.010	1.29
RG*SC	1.42	8.93	0.16	0.875	1.30
C*P	0.246	0.176	1.40	0.167	1.38
C*WC	0.232	0.260	0.89	0.375	1.49
C*RC	0.364	0.179	2.04	0.046	1.43
C*SC	0.45	3.09	0.15	0.885	1.63
P*WC	0.004	0.194	0.02	0.984	1.42
P*RC	-0.046	0.166	-0.27	0.785	1.45
P*SC	-0.06	1.22	-0.05	0.958	1.49
WC*RC	-0.042	0.166	-0.25	0.800	1.44
WC*SC	1.150	0.975	1.18	0.243	1.54
RC*SC	-0.896	0.511	-1.75	0.084	1.79

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.0677795	42.81%	22.26%	0.563758	0.00%

Analysis of Variance for fPARi (pre-grazing) (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.021785	0.021096	0.007032	1.53	0.215
Regression	20	0.198340	0.198340	0.009917	2.16	0.011
Linear	5	0.049376	0.036993	0.007399	1.61	0.170
Quadratic	15	0.148964	0.148964	0.009931	2.16	0.017
RG*C	1	0.000517	0.008062	0.008062	1.75	0.190
RG*P	1	0.003259	0.004928	0.004928	1.07	0.304
RG*WC	1	0.047755	0.054378	0.054378	11.84	0.001
RG*RC	1	0.040505	0.031987	0.031987	6.96	0.010
RG*SC	1	0.000193	0.000116	0.000116	0.03	0.875
C*P	1	0.005920	0.008989	0.008989	1.96	0.167
C*WC	1	0.000664	0.003661	0.003661	0.80	0.375
C*RC	1	0.029188	0.019062	0.019062	4.15	0.046
C*SC	1	0.000084	0.000098	0.000098	0.02	0.885
P*WC	1	0.000108	0.000002	0.000002	0.00	0.984
P*RC	1	0.000196	0.000346	0.000346	0.08	0.785
P*SC	1	0.000005	0.000013	0.000013	0.00	0.958
WC*RC	1	0.000003	0.000296	0.000296	0.06	0.800
WC*SC	1	0.006437	0.006385	0.006385	1.39	0.243
RC*SC	1	0.014130	0.014130	0.014130	3.08	0.084
Residual Error	64	0.294020	0.294020	0.004594		
Total	87	0.514146				

Appendix Q Diversity interaction model output for RUE of swards

Linear model analysis

Estimated coefficient values of RUE (linear model) for monocultures

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0634	0.0436	1.45	0.150	1.50
2	0.0298	0.0436	0.68	0.497	1.50
3	-0.1034	0.0436	-2.37	0.020	1.50
RG	1.4193	0.0803	*	*	1.05
C	1.1205	0.0803	*	*	1.05
P	1.3680	0.0803	*	*	1.05
WC	1.6099	0.0803	*	*	1.05
RC	1.6874	0.0803	*	*	1.05
SC	1.5180	0.0803	*	*	1.05

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.236105	30.87%	23.87%	5.46353	14.24%

Analysis of Variance for RUE (monocultures)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.3455	0.3455	0.11517	2.07	0.111
Regression	5	1.6215	1.6215	0.32429	5.82	0.000
Linear	5	1.6215	1.6215	0.32429	5.82	0.000
Residual Error	79	4.4039	4.4039	0.05575		
Total	87	6.3709				

Quadratic model

Estimated interaction coefficient values of RUE for binary mixtures

Term	Coef	SE Coef	T-Value	P-Value	VIF
Blocks					
1	0.0634	0.0376	1.69	0.097	1.50
2	0.0298	0.0376	0.79	0.432	1.50
3	-0.1034	0.0376	-2.75	0.008	1.50
RG	1.313	0.101	*	*	2.26
C	1.183	0.101	*	*	2.26
P	1.536	0.101	*	*	2.26
WC	1.613	0.101	*	*	2.26
RC	1.558	0.101	*	*	2.26
SC	1.763	0.101	*	*	2.26
RG*C	0.833	0.492	1.69	0.095	1.48
RG*P	-0.562	0.492	-1.14	0.258	1.48
RG*WC	0.363	0.492	0.74	0.463	1.48
RG*RC	0.963	0.492	1.96	0.055	1.48
RG*SC	-0.327	0.492	-0.66	0.509	1.48
C*P	-0.482	0.492	-0.98	0.332	1.48
C*WC	0.113	0.492	0.23	0.819	1.48
C*RC	-0.077	0.492	-0.16	0.877	1.48
C*SC	-1.817	0.492	-3.69	0.000	1.48
P*WC	-0.262	0.492	-0.53	0.597	1.48
P*RC	0.298	0.492	0.61	0.547	1.48
P*SC	-2.102	0.492	-4.27	0.000	1.48
WC*RC	-0.067	0.492	-0.14	0.893	1.48
WC*SC	-0.627	0.492	-1.27	0.208	1.48
RC*SC	0.523	0.492	1.06	0.292	1.48

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.203724	58.31%	43.32%	5.14187	19.29%

Analysis of Variance for RUE (component proportions)

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Blocks	3	0.34551	0.34551	0.115171	2.77	0.048
Regression	20	3.36915	3.36915	0.168458	4.06	0.000
Linear	5	1.62146	0.88729	0.177458	4.28	0.002
Quadratic	15	1.74769	1.74769	0.116513	2.81	0.002
RG*C	1	0.16658	0.11885	0.118848	2.86	0.095
RG*P	1	0.04655	0.05399	0.053990	1.30	0.258
RG*WC	1	0.00927	0.02259	0.022593	0.54	0.463
RG*RC	1	0.09211	0.15882	0.158821	3.83	0.055
RG*SC	1	0.00168	0.01826	0.018263	0.44	0.509
C*P	1	0.00032	0.03971	0.039705	0.96	0.332
C*WC	1	0.03307	0.00220	0.002198	0.05	0.819
C*RC	1	0.00080	0.00101	0.001006	0.02	0.877
C*SC	1	0.43604	0.56481	0.564813	13.61	0.000
P*WC	1	0.00201	0.01172	0.011718	0.28	0.597
P*RC	1	0.04494	0.01523	0.015232	0.37	0.547
P*SC	1	0.78076	0.75593	0.755930	18.21	0.000
WC*RC	1	0.00051	0.00076	0.000761	0.02	0.893
WC*SC	1	0.08618	0.06721	0.067209	1.62	0.208
RC*SC	1	0.04687	0.04687	0.046872	1.13	0.292
Residual Error	64	2.65622	2.65622	0.041503		
Total	87	6.37089				

Appendix R Regression components of RUE and T relationship for monoculture swards in a species mixed pasture diversity experiment at Lincoln University, Canterbury, New Zealand.

Swards	Coefficient value (constant) + SE	Temperature coefficient value + SE	R^2 (Adj)%	P value
PR	0.59 (0.14)	0.05 (0.01)	49	0.01
C	0.61 (0.09)	0.04 (0.01)	59	0.01
P	0.37 (0.24)	0.09 (0.02)	51	0.01
WC	0.79 (0.21)	0.07 (0.02)	42	0.01
RC	0.92 (0.11)	0.05 (0.01)	55	0.01
SC	0.86 (0.27)	0.08 (0.02)	38	0.01

Appendix S Regression components of RUE and T relationship for PR*WC, PR*RC binary mixtures in a species mixed pasture diversity experiment at Lincoln University, Canterbury, New Zealand.

Swards	Coefficient value (constant) + SE	Temperature coefficient value + SE	R^2 (Adj)%	P value
PR*WC	0.79 (0.19)	0.05 (0.01)	34	0.01
PR*RC	0.58 (0.22)	0.08 (0.02)	46	0.01

Appendix T Soil moisture profile for plantain from November 2009 to February 2020 in I8 multi-species pasture mixture experimental site.