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Effect of grazing rotation interval and defoliation residual height on the botanical composition, yield and quality of hyper-diverse pastures.

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ABSTRACT

Pastoral farming systems in New Zealand currently heavily rely on *Lolium perenne* L. (perennial ryegrass)-*Trifolium repens* L. (white clover) (relatively simple) pasture mixtures. However, there are problems with seasonal growth fluctuations, creating periods of feed deficit and surplus. Further, these species result in low nitrogen (N) use efficiency, leading to high N concentrations in urine, N leaching and nitrous oxide (N_2O) emissions. Pressure on these farming systems has grown to improve productivity, efficiency and mitigate environmental impacts. It has been suggested that diverse pastures (those including mixtures of grass, legume and herb species) may provide improved yield, seasonal distribution of pasture supply and quality through the inclusion of species with different characteristics (i.e. heat and drought tolerance, deeper rooting). However, the success of a given pasture mixture is largely dependent on management. Whilst there is a plethora of literature looking into defoliation management of diverse pastures, there is a dearth of studies looking at hyper-diverse pastures (those including >9 species from at least 3 different functional groups). The objectives of this research were to examine how differing defoliation management (i.e. residual height and defoliation interval) impacted the botanical composition, yield and quality of hyper-diverse pasture mixtures. This study indicated that, whilst a large number of species were sown (18 species in the dairy mixture and 19 species in the sheep mixture), less than half were observed to remain present throughout the study period of 7 months (November 2022 to May 2023). Of those present, there were only several that dominated (i.e. perennial ryegrass, white clover, *Plantago lanceolata* L. (plantain) and *Trifolium pratense* L. (red clover), and they were not sensitive to any residual height or interval treatments, over the period of this study. The reversion of the hyper-diverse mixtures to a smaller number of dominant species within a short timeframe poses the question of whether it would have been easier to create a simpler pasture mixture at the outset. However, the survival and dominance of species was undoubtedly driven by edaphic conditions, along with the prevailing climate at time of sowing and throughout the experimental period, and interactions between these and the defoliation management. Overall, there was a general trend where a longer defoliation interval (9 weeks) reduced total yield, whilst shorter intervals (3 and 6 weeks) increased yields in association with higher residual heights. There were no significant differences in nutritive value parameters, but there were trends in the data which showed that higher residual heights and longer rotations resulted in increased lignin and neutral detergent fibre and decreased metabolisable energy for these pastures during May. However, regardless of treatment, the nutritive value parameters showed that these hyper-diverse pasture mixtures are capable of maintaining high animal performance. More research needs to be undertaken

before clear recommendations are able to be made regarding likely suitable situation-specific mixtures, and these will vary with region and management system (e.g. dairy, sheep, and beef). Further research should focus on how to achieve successful establishment of a large number of species in mixture, and then to define the optimal defoliation management that will maintain diversity over a longer time period. Overall, the results of this study indicated that hyper-diverse pasture mixtures can be defoliated under a range of residual heights and intervals whilst maintaining yield and quality, although with less diversity, suggesting that these mixtures have the potential to be valuable in New Zealand pastoral systems.

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CHAPTER 1. GENERAL INTRODUCTION

Temperate pastoral systems, such as those in New Zealand (NZ), are traditionally based on grass/clover mixtures, predominantly sown with perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Kemp et al., 1999). Extensive research has identified the pasture management required to optimise production, quality, and persistence of these species (Kemp et al., 2000; Fulkerson and Donaghy, 2001; Goh and Bruce, 2005; Mapp et al., 2013; Catalano et al., 2020; Donaghy et al., 2021). However, a major limitation of ryegrass/white clover pastures is its fluctuation in seasonal growth, creating periods of feed deficit and surplus (Vibart et al., 2016), along with a strong dependence on nitrogen (N) inputs to achieve high yields, especially when clover content it low (Baker et al., 2023a). This fluctuation in growth, especially poor growth during warmer, drier periods, has the potential to impact feed intake (Fulkerson and Donaghy, 2001) and animal performance (Hendriks et al., 2017).

The current increased frequency, and higher future likelihood, of warmer, drier, and more varied extreme weather events under climate change scenarios, has led to strong interest from the NZ pastoral sector in the use of alternative herbage species and more diverse pasture mixtures, along with research on the management, quality, composition, and animal performance from these mixtures (Vibart et al., 2016; Donaghy et al., 2021; Sheridan et al., 2022b). However, while some guidelines exist (i.e. grazing residual height and/or defoliation interval of some species or mixes) (Edwards et al., 1996; Cranston et al., 2015b; Baker et al., 2023c), these do not account for ’hyper-diverse’ pasture mixtures that have multiple species from a number of functional groups (i.e. grasses, legumes and forage herbs). In more recent years, these ‘hyper-diverse’ pastures have become more widely promoted and used, however, scant research has been published on this (Bell et al., 2020).

In 2022, the NZ Ministry for Primary Industries funded the ‘Whenua Haumanu’ program, which is the most comprehensive research program investigating pastoral agriculture, including regenerative management, in NZ (MPI, 2022). As part of this 7-year program, ‘standard’ ryegrass/clover pastures (as are sown by the majority of farmers) are being evaluated alongside ‘hyper-diverse’ pastures (19-21 different plant types/species, recommended by regenerative consultants). The current thesis will examine the effect of differing frequencies and residual heights of defoliation on the botanical composition, yield and herbage quality of two ‘hyper-diverse’ pasture mixes (sheep and dairy), compared with ‘standard’ ryegrass/clover pastures, over a period of 27 weeks.

CHAPTER 2. REVIEW OF LITERATURE

2.1. Introduction

Internationally, farming systems are facing increasing pressure to improve productivity and sustainability, and to mitigate environmental impacts. Temperate farming systems, such as those in New Zealand (NZ), southern Australia, and Ireland, are heavily reliant upon pastoral grazing to provide an economic advantage (Hanson et al., 1998; Dillon et al., 2005; Ramsbottom et al., 2015). Historically, pasture-based systems in NZ have been predominantly sown with perennial ryegrass (*Lolium perenne* L.) - white clover (*Trifolium repens* L.) based mixtures (Charlton and Stewart, 1999; Kemp et al., 1999). This provides a year-round feed supply, with varying levels of imported supplementary feed (Roche et al., 2017b). This sown pasture mixture provides a low-cost, high-quality, and highly productive feed that grows in a range of conditions with well-documented best-management practices defined and outlined (Daly et al., 1996; Fulkerson and Donaghy, 2001; Vibart et al., 2016; Macdonald and Roche, 2023). However, there are problems with seasonal growth fluctuations, creating periods of feed deficit and surplus (Vibart et al., 2016), along with a strong dependence on nitrogen (N) inputs for the production achieved (Baker et al., 2023b). Furthermore, these species can result in low N use efficiency, leading to high urinary N concentrations, N leaching, and elevated greenhouse gas (GHG) emissions (Carmona-Flores et al., 2020).

Climatic conditions can have a large and varied impact on pastoral production systems. Whilst perennial ryegrass-white clover pastures can grow under a range of conditions, climate change has resulted in an increase in the severity and frequency of adverse environmental conditions (i.e. drought, floods, and heatwaves) (Farrow et al., 1993; Bodeker et al., 2022). This introduces an increased risk for pastoral farmers, as there is greater seasonal variability in weather events, which can negatively affect both the yield and persistence of pastures (Donaghy et al., 2021). Due to perennial ryegrass-white clover being the predominant sown pasture mixture in NZ, and the increasing occurrence of adverse climatic conditions, the failure of this basic pasture mix to adapt to climate change has caused significant concern for NZ pastoral farmers (Donaghy et al., 2021).

Growing environmental concerns have driven the search for alternative solutions to minimise both the impact of climate change on pastoral systems, and the impact of agriculture on the wider environment (Beukes et al., 2014; Sheridan et al., 2022a). 'Diverse' pastures (also referred to as multispecies swards), are comprised of at least three functional groups (i.e. grass, legume, and forage herbs), with a minimum of one species from each (Vibart et al., 2016). These diverse pastures can potentially reduce seasonal variability in herbage quality (Grace et al., 2019b; Carmona-Flores et al., 2020; Schaub et al., 2020b) and increase pasture yield (Woodward et al., 2013; Pembleton et al., 2015; Distel et al., 2020), and thus be expected to improve animal production (Carmona-Flores et al., 2020; McCarthy et al., 2020; Nakajima et al., 2022) and reduce environmental impacts (Distel et al., 2020; Cummins et al., 2021). The degree to which these mixtures are advantageous under NZ conditions is often ambiguous, limiting their potential implementation. Furthermore, there is limited published scientific data regarding diverse pasture systems; specifically, a lack of published data on the establishment, persistence, and management of diverse pasture mixtures in comparison to ryegrass-white clover pastures. It is essential to evaluate the potential advantages and disadvantages, and to outline the necessary steps for the successful integration, of diverse pasture mixtures into farm systems throughout NZ.

This literature review will explore diverse pastures compared to 'simple' (i.e. ryegrass-clover) traditional NZ planted pastures. The potential impact on pasture yield, botanical composition, herbage nutritive value, grazing management, animal production, and environmental impact will be investigated. Additionally, limitations of current knowledge will be identified.

2.2. What is diverse pasture?

The term diverse or multispecies pasture lacks a widely accepted single definition. Various definitions have been proposed throughout the literature, reflecting different perspectives and criteria for classifying pastures as diverse. Historically, diverse pastures have been defined as a complex mixture of pasture species, balancing dominant and subordinate plants (Campbell, 1990). Another definition refers to diverse pasture as a "mixture of more than one alternative grass and clover species" (Moloney, 1991; Charlton and Stewart, 1999). In recent literature, the definitions of diverse pasture range from vague to more specific requirements. Some definitions emphasise the inclusion of a range of grass, legume, and forage herb species (Bell et al., 2020), a "retained high level of species diversity" (Woodward et al., 2013), or incorporating alternative grass species within more common mixtures (Nobilly et al., 2013; Beukes et al., 2014; Vibart et al., 2016). More specific requirements include a minimum of at least three sown plant species (Pembleton et al., 2015; Vibart et al., 2016; Carmona-Flores et al., 2020), or at least two different functional groups (Grace et al., 2018; Cummins et al., 2021; Baker et al., 2023b). The common theme throughout these definitions has been the inclusion of a range of species from multiple functional groups, and often with different environmental niches, compared with the more commonly sown simple pasture mixture (e.g. perennial ryegrass-white clover).

The terms simple or ‘standard’ pasture have often been used to describe a pasture mixture with few species that are widely sown in pastoral systems (Vibart et al., 2016; Romera et al., 2017; Distel et al., 2020). These mixtures have been described as having “limited diversity” (Distel et al., 2020) and usually only contain two species (Nobilly et al., 2013; Carmona-Flores et al., 2020). In NZ, as previously mentioned, the predominant simple planted pasture consists of perennial ryegrass and white clover (Beukes et al., 2014; Vogeler et al., 2017). This pasture mixture is widely used due to its high nutritional value, quick establishment, reasonable productivity, and well-understood grazing management (Totty et al., 2013; Bell et al., 2020). Furthermore, perennial ryegrass is resilient under a range of grazing residual heights (Donaghy et al., 2021) and environments in NZ (Charlton and Stewart, 1999), except for areas with extreme environmental conditions (Kemp et al., 1999). Yields of these pasture systems can be further increased with the addition of N fertiliser and irrigation, allowing farms to achieve economically optimum levels of pasture utilisation (Pembleton et al., 2015).

The main difference between simple and diverse pastures is the number of functional plant groups within the pasture (Distel et al., 2020; Cummins et al., 2021; Grange et al., 2021; Baker et al., 2023c). Diverse pastures contain many species of differing characteristics, which relate to the functional plant group (legume, herb, and grass), growth patterns/habits, optimum environmental conditions, nutritional value and grazing management requirements (Vibart et al., 2016; Distel et al., 2020). While there is a substantial amount of research on perennial ryegrass and white clover (Roche et al., 2017a), resulting in well-established management practices, there is relatively less research on diverse pastures and the interactions between species within these pastures.

It is interesting to follow the development and history of NZ pastures. Early settlers in NZ in the 1800s followed European trends in using multi-species pasture mixtures in the form of ‘shotgun mixtures’, containing at least 20 pasture species (Campbell, 1990; Charlton, 1991). Arguments in favour of these mixtures reported reduced seasonal variation, higher nutritive value, and a higher chance of some species being suitable for a range of conditions, which were “not so widely known”, according to Alexander (1933). In the 1930s the breeding and seed production of ryegrass and white clover led to the widespread use of simple mixtures, as demand increased for temporary pastures that could provide quick returns and fit in with crop rotations (Alexander, 1933; Charlton and Stewart, 1999). This has led to the common use of simple pasture mixtures predominantly ryegrass-white clover, in NZ pasture-based systems.

Perennial ryegrass and white clover pastures are productive under a wide range of grazing management, provided that water and temperature are within the optimal range for temperate species (Brown et al., 2022). Early breeding and research efforts focused on perennial ryegrass, resulting in more scientific knowledge on this species compared to all others. However, as other species were researched, the more common species in NZ came to encompass a narrow range of ecological conditions – requiring medium-high fertility and moderate grazing intensity – based on the success experienced by a large number of farmers (Campbell, 1990). Up until the 1990s, there had been a large focus on research into perennial ryegrass and white clover, during which farmer adoption of diverse pasture mixtures was influenced more by “fashion” than science (Charlton, 1991). Furthermore, minimal research had been undertaken on the complementarity of species within mixes (Johnson et al., 1994).

Due to the lack of published scientific data in the early 1990s, the use of diverse pasture mixtures was small, but reportedly common among NZ organic farmers (Daly et al., 1996). Anecdotal reports from farmers who utilised complex pasture mixtures suggested improved animal performance, drought tolerance, persistence, reduced health disorders, and more uniform seasonal growth patterns compared to simple pastures; however, there was a lack of published scientific evidence supporting these claims (Daly et al., 1996). At this time, studies suggested that diverse pasture mixtures were better utilised on less-fertile soils where simple pasture mixtures weren’t successful, as under conditions of higher soil fertility, only one or two species were likely to dominate (Charlton and Stewart, 1999).

2.3. Pasture production and growth trends

From this point forward in this thesis, simple pastures will be defined as mixtures of two to three common grass and legume species only (e.g. perennial ryegrass and one or two clovers). While the primary example used in NZ is the perennial ryegrass-white clover based mixture, it is important to note that there are other variations of simple pastures that exclude these species. As there is no widely used definition of diverse pastures, any discussion regarding results from other literature will be based on their respective definitions of diverse.

2.3.1. Simple pastures

In addition to the ‘standard’ perennial ryegrass-white clover pastures previously discussed, other simple pasture mixes commonly found in NZ include those containing tall fescue [*Festuca* *arundinacea* (Schreb.)], cocksfoot (*Dactylis glomerata* L.), phalaris (*Phalaris aquatica* L.), and red clover (*Trifolium pratense* L.). These species all have more active growth (defined as kg dry matter (DM)/ha/day) during periods of warmer, drier weather (summer and autumn in NZ) in comparison to perennial ryegrass-white clover mixtures (Johnson et al., 1994; Kemp et al., 1999). These species have slightly different seasonal distributions of yield (or production, defined as kg DM/ha) (refer to Table 1) and this sometimes impacts on annual yield (refer to Table 2).

Table 1: Seasonal yield [percentage (%)] of four grass and two clover species grazed with dairy cows in Taranaki, NZ [adapted from Roberts and Thomson (1984); Judd et al. (1990)]. Each column sums to 100%.

	Perennial ryegrass[1]	Tall fescue[1]	Cocksfoot[1]	Phalaris[1]	White clover[2]	Red clover[1]
Winter	12	11	12	12	5	-
Spring	40	36	34	31	34	30
Summer	33	35	36	37	43	50
Autumn	15	18	18	20	18	20*

*This data was provided as combined yield for winter and autumn seasons

[1]Judd et al. (1990)

[2]Roberts and Thomson (1984)

Table 2: Annual yield (t DM/ha) of perennial ryegrass (Kemp et al., 1999), tall fescue, cocksfoot, Phalaris (Moloney, 1991), white clover (Brock and Hay, 2001) and red clover (Brown et al., 2003) in NZ.

	Perennial ryegrass	Tall fescue	Cocksfoot	Phalaris	White clover	Red clover
Annual yield (t DM/ha)	10-25	20	13	18	10-12	16

The major limitation to pasture growth in summer is low soil moisture levels, although higher temperatures also play a role in parts of NZ, whereas in winter, growth is limited by low temperatures, although high soil moisture levels (i.e. waterlogging) may also impact growth (Kemp et al., 1999). The poor performance of perennial ryegrass under dry conditions is partially due to the relatively shallow roots of this species (Vogeler et al., 2017), with 75% of roots at 0-7cm depth of soil (Wedderburn et al., 2010).

It is suggested that pastures with fewer numbers of species are associated with easier establishment and grazing management, as each species can be considered (Kemp et al., 1999).

The limitation of seasonality in growth and yield is that the periods of feed shortage and surplus need to be managed to ensure that animal requirements are met, which can increase the cost of production (Pembleton et al., 2015). However, simple pastures such as perennial ryegrass-white clover are relatively well understood, which allows for the manipulation of the pastoral system to stabilise or increase yield. For example, irrigation can improve pasture growth over moisture-limited months, and N fertiliser can be used to enhance the overall yield (Pembleton et al., 2015). Additionally, optimal soil fertility management practices are well established for these mixtures, further contributing to improved yield.

However, research suggests that perennial ryegrass is heavily dependent on N fertiliser to improve yield and doesn't persist across all temperate regions (Harris et al., 1996; Grace et al., 2019a). Therefore, there is interest in looking toward other options, such as diverse pastures, to maintain higher productivity whilst reducing reliance on N fertilisers.

2.3.2. Diverse pastures

Whilst simple pastures have been successful in most temperate pastoral regions of the world, there has been an interest in research into alternative pasture species for environments not fully suited to perennial ryegrass-based pastures (Vibart et al., 2016). Research into a larger number of pasture species has increased options when designing diverse pastures and their suitability to differing environments (Distel et al., 2020), contrasting the shotgun approach in earlier years (Alexander, 1933), where multiple species were sown and then the fittest or most adaptable survived. The inclusion of species from different functional groups has the potential to improve the stability of seasonal production and therefore overall yield, by capitalising on differences in species' tolerance to environmental and biological disturbances (Distel et al., 2020). Furthermore, the presence of multiple species offers the potential to exploit temporal and spatial niches within pasture, taking advantage of variations in soil types and environmental conditions (Pembleton et al., 2015). With differences in rooting depth (Vibart et al., 2016), optimal temperatures and nutrient requirements, multiple species are theoretically able to coexist, however, this depends on the type of species selected and their interactions with one another.

Studies show large discrepancies in the amount of annual DM yielded by diverse pastures (see Table 3). Compared to simple pastures, diverse pastures have either increased yields (Sanderson et al., 2004; Romera et al., 2017; Baker et al., 2023c), no significant difference in yield (Woodward et al., 2013; Edwards et al., 2015; Grace et al., 2018; Carmona-Flores et al., 2020) or in few cases, decreased yield (Beukes et al., 2014) (see Table 3). Increased yield when comparing diverse with simple pastures could be explained by a greater number of species utilising specific spatial or temporal niches (Davis et al., 2000; Sanderson et al., 2004). However, these niches are site-specific and species-dependent, and it may require several years and larger areas for their full exploitation, which is often overlooked in short-term plot studies (Tilman et al., 2001; Beukes et al., 2014). Some studies suggest that this increase is more likely due to specific species and increased functional diversity, rather than the total number of species present (Distel et al., 2020; Grange et al., 2021; Baker et al., 2023c). This could explain the yield advantage in moisture-limited periods from the inclusion of deep-rooted herbs e.g. chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) in the diverse mixtures, compared to simple pastures (Vibart et al., 2016). In contrast to the recorded higher yields, the lower yields that were recorded in other studies were primarily associated with lower pasture growth rates over winter (Beukes et al., 2014). It is also important to note that the reduction in annual yield, as reported by Beukes et al. (2014), was derived from a model based on only two pasture studies, both located in the Canterbury region of NZ .

Diverse pastures often include species that are more heat- and drought-tolerant, resulting in increased growth during the summer and autumn months (Sanderson et al., 2004; Pembleton et al., 2015). This is supported by many studies (Woodward et al., 2013; Romera et al., 2017; Vogeler et al., 2017), that included more deep-rooted species e.g. lucerne (*Medicago sativa* L.) and chicory, which were able to withstand water limitations (Pembleton et al., 2015). However, as previously mentioned, this increased summer growth is often balanced by reduced growth over the cooler months compared to ryegrass-based pastures, as ryegrass has reduced sensitivity to cooler temperatures (Vogeler et al., 2017). These differences in seasonal pasture growth across the year can result in smaller feed shortages and surpluses. Therefore, it is likely that diverse pastures have a more even seasonal distribution of pasture growth, even if there is no impact on annual DM yield compared with ryegrass-based pasture

Table 3: Effect of diverse versus simple pasture mixtures on annual yield [kg of dry matter (DM) per hectare] and seasons observed.

Source	Study	Period	Location	Simple pasture species	Additional species in diverse pasture	Difference in annual yield	Seasons difference observed
Baker et al. (2023c)	Field study	1.5 yr	Dublin, Ireland	PR + WC	Timothy (*Phleum pratense* L.), red clover (*Trifolium pratense* L.), chicory (Cichorium intybus *L.*), plantain (*Plantago lanceolata* L.)	16.8% increase	S
Beukes et al. (2014)	Model	2 yr	Canterbury & Waikato, NZ	PR + WC	Prairie grass (*Bromus catharticus* Vahl.), red clover, chicory, plantain, lucerne (*Medicago sativa* L.)	9.9% decrease	S + A
Carmona-Flores et al. (2020)	Field study	21 d	Oregon, USA	PR + WC	X festulolium, chicory, plantain, bird's-foot trefoil (*Lotus corniculatus*)	2.6% increase*	
Edwards et al. (2015)	Field study	9 d	Canterbury, NZ	PR + WC	Plantain, chicory, lucerne	0.25% increase*	S
Grace et al. (2018)	Field study	1.5 yr	Dublin, Ireland	PR + WC	Timothy, red clover, chicory, plantain	6.2% increase*	S + A
Moloney (1991)	Field study	4 yr	Waikato, NZ	PR + WC	Tall fescue [*Festuca arundinaceum* (Schreb.) Wimm], chicory, cocksfoot (*Dactylis glomerata* L.)	20.1% increase	S
Nobilly et al. (2013)	Field study	2 yr	Canterbury, NZ	PR + WC	Prairie grass, red clover, chicory, plantain	10% increase	S
Romera et al. (2017)	Model	3 yr	Waikato, NZ	PR + WC	Chicory, plantain, red clover, lucerne, prairie grass, timothy	5.6% increase	S + A + W
Sanderson et al. (2005)	Field study	2 yr	Pennsylvania, USA	Cf + WC	Chicory, tall fescue, Kentucky bluegrass (*Poa pratensis*), red clover, bird's-foot trefoil, lucerne	43% increase	Dry year
Vogeler et al. (2017)	Model	2 yr	Canterbury, NZ	PR + WC	Phalaris (*Phalaris aquatica* L.), lucerne, tall fescue, browntop (*Agrostis capillaris*)	2.7% increase	S
Woodward et al. (2013)	Article	3 yr	Waikato, NZ	PR + WC	Prairie grass, chicory, plantain, lucerne	None	S + A

PR + WC = perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Cf + WC = cocksfoot and white clover. S= summer. A = autumn. W = winter.
*Not statistically significant

2.4. Pasture composition

Monitoring the botanical composition of pastures can identify changes in dominant species and proportions of functional groups over time. This is mostly influenced by environmental conditions and grazing management (Belesky et al., 2002a; Belesky et al., 2002b). These changes are important, as they may influence pasture quality and grazing management (Crosthwaite et al., 1996; Belesky et al., 1999; Sleugh et al., 2000; White et al., 2004).

2.4.1. Simple pastures

It has been suggested that with simple pastures, the majority of herbage production is from the grass species (Kemp et al., 1999). In NZ, it is common to find that the botanical composition is comprised of approximately 80-90% of perennial ryegrass (Chapman et al., 1995; Charlton and Stewart, 1999; Mapp et al., 2013). Since most simple pastures are made up of a single legume and grass species, it is important to consider the growth patterns of the two species to be able to manipulate grazing management to the dominant species at a particular time. Whilst ryegrass is dominant during spring (Kemp et al., 1999), clovers are generally dominant during late-summer (Brock et al., 2000). Cocksfoot and tall fescue are dominant during summer and winter, while phalaris is dominant during autumn and early-winter (Stewart, 2022).

A study by Baker et al. (2023c) showed that the percentage of ryegrass in a ryegrass-white clover pasture decreased by 35% from spring to autumn in a two-year study (see Table 4). Over the same period, the legume content increased by 28%. Similarly, Hearn et al. (2022) found a significant reduction in grass content from spring to autumn (48%) along with a significant increase in the legume content over the same period (47%). However, Grace et al. (2018) showed no significant seasonal change in grass or legume content. This could be explained by the lower than intended legume levels in the mixture after establishment.

Table 4: Botanical composition [percentage (%)] of perennial ryegrass (*Lolium perenne* L.)-white clover (*Trifolium repens* L.) pastures, seasonally.

Source	Spring		Summer		Autumn	
	PRG	**WC**	**PRG**	**WC**	**PRG**	**WC**
Hearn et al. (2022)	93.2	6.0	56.9	39.1	45.2	53.0
Baker et al. (2023c)	92.0	5.0	57.0	32.0	57.0	33.0
Grace et al. (2019a)	91.0	6.0	94.0	5.5	92	8

PRG= perennial ryegrass. WC= white clover

Establishment is a critical period of the life cycle for many plants (Tilman, 2004), as this affects the future production and persistence of individual pasture species (Hampton et al., 1999). The success of establishment is majorly influenced by ease of resource acquisition, which can be affected by the timing of germination since the availability of resources varies seasonally (Shayanfar et al., 2020). Perennial ryegrass is commonly regarded as being the fastest-establishing perennial grass species used in NZ (Kemp et al., 1999). Tall fescue-based pastures have been recorded as slower to establish when compared to perennial ryegrass (Easton et al., 1994; Lowe et al., 2000; Kularatne and Lawson, 2002; Nie et al., 2004; Tharmaraj et al., 2008; Tozer et al., 2017). This could be due to a lower base temperature being required for emergence in the field, faster germination rates, root elongation, tillering and regrowth after grazing (Brock et al., 1982; Moot et al., 2000). An option for pastures based on grasses other than ryegrass is to look into different timing of sowing (i.e. spring vs autumn) (Tharmaraj et al., 2008). The emergence of white clover is relatively slower than ryegrass (Shayanfar et al., 2020). To improve the establishment of white clover, it has been recommended to use a seeding rate of two to five kg/ha (Frame and Newbould, 1986; Brock and Kane, 2003). Although, some studies have shown, that even with this recommended seeding rate, legumes did not establish to target levels, regardless of diversity (Grace et al., 2018).

From seedling to the first production year, there have been reports of a 55.96% and 84.31% reduction in the grass-to-legume ratio in ryegrass-white clover mixtures (Catalano et al., 2020; Baker et al., 2023c), respectively. Other studies have shown a reduction in the proportion of clover in pasture over a longer period (Daly et al., 1996; Brock and Hay, 2001; Sanderson et al., 2005). Furthermore, Murray et al. (2022) supported this over a four-year field trial with eight different cultivars of perennial ryegrass. Even though there is evidence supporting ryegrass as having greater establishment, Tozer et al. (2017) found that tall fescue-based pastures had greater persistence compared to ryegrass, especially in dry-summer conditions (Easton et al., 1994).

Weed ingress is an important part of pasture composition, as it affects the quality and yield of pasture production (Connolly et al., 2018) by diverting available resources (light, water, and nutrients) to undesirable species and providing competition to sown species (Grice and Campbell, 2000; Oerke and Dehne, 2004). The control of weed growth is important as it provides a major source of inefficiency (Oerke and Dehne, 2004; Connolly et al., 2018), and can reduce pasture utilisation and persistence (Tozer et al., 2011).

There has been evidence to suggest that grass-legume mixtures have a lower and more stable weed proportion compared to perennial ryegrass monocultures (Connolly et al., 2018). The inclusion of legumes in pastures has also been linked to an increase in weed suppression due to an associated reduction in N fertiliser required for pasture growth (Kemp et al., 2010; Lüscher et al., 2014; Baker et al., 2023b). However, grass species are generally more suppressive against weeds, as they result in a higher ground cover density compared to legumes (Wardle et al., 1995), and thus better competition against weed seedlings (Hartley and Thai, 1979; Ivens and Mlowe, 1983). Ryegrass-based pastures have a higher weed incursion after dry autumns (Musgrave and Daly, 2004), and lower tolerance to insect pests when endophyte-free (i.e. ASW, black beetle, grass grub) (Easton et al., 1994; Zydenbos et al., 2011) compared to other species such as tall fescue. Looking into alternative grass species (i.e. cocksfoot and tall fescue) has shown a reduction in weed proportions, as they have better ground cover compared to ryegrass (Musgrave and Daly, 2004; Tracy and Sanderson, 2004). Furthermore, Tozer et al. (2017) reported a 25% reduction in broadleaf weed occurrence in tall fescue-based pastures compared to ryegrass. However, there is a big impact of weed control before sowing and within the first year (Tozer et al., 2011; Tozer et al., 2017). For simple pastures, chemical weed control is standard management, with selective and broad-spectrum herbicides available that won't damage grasses or legumes (Power et al., 2013; Ghanizadeh and Harrington, 2019).

2.4.2. Diverse pastures

Diverse pastures can be thought of as having at least three functional groups with at least one species from each group (Vibart et al., 2016). The proportion of these groups within the pasture changes both annually and seasonally; for example, the content of a number of legumes increases in spring, and decreases in autumn and summer, the content of cool-season grasses is highest in autumn through winter (Baker et al., 2023c), and the content of forage herbs generally increases over summer (Woodward et al., 2013; Baker et al., 2023c).

Table 5: Botanical composition [percentage (%)] of diverse pastures over two years containing grasses, legumes, and herbs.

Source	Grass (%)		Legume (%)		Herb (%)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Baker et al. (2023c)	18	30	39	43	40	20
Grace et al. (2018) 6-species	73	85	7	7	21	7
Grace et al. (2018) 9-species	79	89	4	4	17	7

In studies with diverse pastures, the grass fraction was reported to increase by approximately 10-12% per year, while the legume fraction increased by approximately 0-4% per year (Grace et al. 2018; Baker et al. 2023) (see Table 5). There were also reports of the herb fraction decreasing by 10-20% per year (Grace et al., 2018; Baker et al., 2023c). A similar reduction in plantain is also reported by Bryant et al. (2019), Rodriguez-Firpo et al. (2022), Hearn et al. (2022), and Nguyen et al. (2022). The reduction in herbs (plantain and chicory) in these experiments can partially be explained by selective grazing (Jing et al., 2017; Grace et al., 2018), which can potentially be reduced by altering grazing management (Grace et al., 2018). However, regardless of their management, chicory and plantain are relatively short-lived plants, making them susceptible to persistency issues over time (Li and Kemp, 2005; Grace et al., 2018; Baker et al., 2023c).

As previously mentioned, seedling establishment is a critical period in the plant lifecycle, affecting pasture production and weed competition, in turn impacting the long-term productivity of the pasture (Hampton et al., 1999; Tilman, 2004; Dillehay et al., 2011). The time of sowing is an important factor for establishing diverse pastures, as optimum germination conditions may differ between species and functional groups. The ideal time for sowing most grass and legume species in NZ is early-autumn or late-spring, as soil temperature is near optimum (Hampton et al., 1999). Although, soil moisture may be a limiting factor for autumn sown pastures (Hampton et al., 1999). It is suggested that ryegrass has rapid germination and seedlings may outcompete slower-establishing species such as chicory (Moot et al., 2000) and plantain (Powell et al., 2007) , especially under high-fertility conditions (Tozer et al., 2016). Spring sowing is recommended for chicory and plantain rather than autumn, as grass regrowth is delayed, preventing existing pastures from outcompeting establishing herbs for both resources and light (Sanderson and Elwinger, 2000). It is also suggested to delay grazing until the six-leaf stage for plantain and the seven-leaf stage for chicory to minimise plant loss (Powell et al., 2007; Raedts and Langworthy, 2020). Furthermore, when sown in autumn, chicory goes through winter vernalisation, inducing reproductive growth (stem and flower growth), while with spring sowing plants usually remain vegetative until the second summer (Stewart, 2022).

As previously mentioned, anecdotal reports from farmers are that diverse pastures are more persistent than simple pastures, however, the persistence of individual species and groups within the diverse pasture can be an issue, especially the herbs within the pasture. The issue of the persistence of individual species within a diverse pasture has been studied by several authors e.g. Sanderson et al. (2005); Deak et al. (2007); Romera et al. (2017); Jaramillo et al. (2021), who suggest that diverse pastures may require a higher renewal rate compared to simple pastures, to maintain the advantages of species diversity. For example, plantain, and chicory have an average lifespan of 2-7 years (Li et al., 1997b; Belesky et al., 1999; Ayala et al., 2011), which is mainly dependent on the suitability of the environmental conditions, establishment and grazing management (Mangwe et al., 2020). Furthermore, it has been suggested that plantain and chicory, under intensive rotational grazing is unable to persist through means of natural reseeding (Raedts and Langworthy, 2020).

The management of pastures will have a significant impact on how competitive the dominant species are, and thus which species are dominant both seasonally and annually. However, some studies suggest, no matter what the grazing management, that the long-term outcome will be the same, with a continual reduction over time in species/functional diversity (Sanderson et al., 2005; Distel et al., 2020). This can be partly explained by an increased grazing selection toward the forage herb and legume components of the pasture (Jaramillo et al., 2021; Hearn et al., 2022). It has been suggested that diverse pastures would be more successful in maintaining species diversity in areas with lower soil fertility, allowing for more opportunities for niche differentiation/less-dominant species to establish and grow (Campbell, 1990).

Table 6: Percentage (%) of weeds in simple and diverse pastures.

Source	Diverse pasture weed (%)	Simple pasture weed (%)
Carmona-Flores et al. (2020)	0.4	1.6
Tracy and Sanderson (2004)	3	4
Connolly et al. (2018)	7	32
Woodward et al. (2013)	4	7
Tozer et al. (2017)	13	16

Many studies have shown a reduction in weed ingress when comparing diverse and simple pastures (see Table 6). It has been suggested that sowing a greater number of species is associated with a reduction in weed ingress in NZ (Dodd et al., 2004; Tozer et al., 2004; Tharmaraj et al., 2008; Woodward et al., 2013) and elsewhere (Tilman et al., 1996; Tracy and Sanderson, 2004; Connolly et al., 2009; Finn et al., 2013). However, this result may be due to several reasons. One theory is that with increased species diversity, there is a higher likelihood of spatial and temporal differences in the environment being filled with species with different functionality, increasing ground cover and thus reducing the incursion of weeds through greater competition (Dodd et al., 2003; Sanderson et al., 2005; Diaz and Cabido, 2009; Jing et al., 2017). Some suggest reduced weed ingress is a result of the diversity of functional groups present, exhibiting functional traits that would have been filled by weeds (Dodd et al., 2003; Vibart et al., 2016; Tozer et al., 2017). Other studies suggest that it is the more effective capture of light in diverse pastures that is the important mechanism for weed suppression (Renne et al., 2006; Frankow-Lindberg, 2011; Sanderson et al., 2012). Husse et al. (2016) states that under intensively managed pastures with more than 1.5t DM/ha yield per harvest, more than 95% of light incidence is captured. This leaves negligible light and less space at ground level for weed development (Soder et al., 2007). However, this suggests that under less productive pastures, with more sparse canopy closure, increased penetration of light could promote weed development at ground level (Connolly et al., 2018). Another theory is that increased diversity is correlated to an increase in resource acquisition, making the pasture more resistant to invasion by unsown species, as these resources are more scarce (Tilman, 1999; Sanderson et al., 2005; Vibart et al., 2016; Connolly et al., 2018). However, the increased total biomass associated with diverse pastures can make it difficult to identify the specific mechanisms leading to weed suppression (Tracy and Sanderson, 2004).

Reduced weed incursion can also be related more to specific species present, rather than increased species diversity (Crawley et al., 1999; Deak et al., 2007; Sanderson et al., 2007; Soder et al., 2007), and this is due to the individual species occupying the same, or similar, ecological niche as the weeds (Deak et al., 2007; Vibart et al., 2016). Although, the extent to which weeds are suppressed is dependent on how competitive the dominant species within the pasture are (Smith et al., 2004). As a result, any disturbance to the presence or competition of such species (i.e. during establishment) could result in weed incursion (Pembleton et al., 2015). Therefore, while many studies have shown a reduction in weed incursion in diverse pastures, many factors could potentially impact these results.

It has been suggested by many studies that herbs may occupy the niche of broadleaf weed species, improving pasture utilisation (Musgrave and Daly, 2004; Tozer et al., 2016; Jaramillo et al., 2021). It has also been suggested that pastures based on tall fescue have reduced weed proportions compared to ryegrass and brome grass (Tilman et al., 1996; Musgrave and Daly, 2004; Tracy and Sanderson, 2004). Although, these claims of one species being better at reducing weed ingress need more data with a larger range of environmental conditions to be validated (Tozer et al., 2009). It is also likely that other factors (i.e. intensive grazing management) plays a large role in weed ingress (Sanderson et al., 2007; Pembleton et al., 2015), resulting in desirable species being weakened and lost over time through preferential grazing (Rutter et al., 2004; Soder et al., 2007; Jaramillo et al., 2021).

In contrast to the positive impact of pasture diversity on generally reducing weed ingress, once weeds establish, their control in diverse pastures can be a challenge whilst maintaining diversity within the pasture. Currently, standard management for controlling unsown weed species within established pastures is through chemical control, utilising a range of both broad-spectrum and targeted herbicides (Baker et al., 2023b). However, at present, there are no post-emergence herbicides that are both broad-spectrum and safe for mixtures of grasses, legumes, and herbs (Pembleton et al., 2015; Ghanizadeh and Harrington, 2019; Jaramillo et al., 2021; Baker et al., 2023b). Even though many studies show a reduction in weed incursion with multi-species pastures, there are no control options for post-emergence weed management that are not labour intensive, for example, spot spraying or weed wiping individual weeds with herbicide, physical removal of weeds through pulling or cutting, regular mowing of areas to prevent seed set (Ghanizadeh and Harrington, 2019; Baker et al., 2023b). Farmers are concerned about this, and the lack of advice regarding these issues, as weed incursion could negatively impact species diversity, especially in pastures with species from multiple functional groups (Pembleton et al., 2015; Grace et al., 2018).

Despite the number of studies showing positive impacts of diverse pastures on weed suppression, this evidence is mostly from plot studies. Results from mown plot studies under uniform defoliation may not accurately represent results under livestock grazing with animal selectivity, and urine and faecal patches (Sanderson et al., 2005; Tozer et al., 2017). Therefore, the long-term persistence of these diverse pastures is yet to be fully explored (Vibart et al., 2016; Ghanizadeh and Harrington, 2019).

2.5. Pasture quality

Pasture quality (or nutritive value) is heavily influenced by botanical composition and pasture management, especially grazing management and fertiliser application. The composition of multi-species pastures shows large variability over time and is mainly influenced by grazing management and the environment (Belesky et al., 2002a; Belesky et al., 2002b). These shifts in botanical composition may significantly influence quality, as proportions of functional groups within the pasture change i.e. legume and grass proportions having a large influence on crude protein (CP) and neutral detergent fibre (NDF) concentrations, respectively (Deak et al., 2007; Sanderson, 2010; Jing et al., 2017). It is also suggested that to see a significant difference in nutritive value, the legume component needs to make up at least 30% of the pasture in diverse pastures, which is difficult to maintain (Harris and Thomas, 1973; Grace et al., 2018). Grazing management also plays a large role, as it can both directly and indirectly affect quality through plant growth characteristics (i.e. stem-to-leaf ratio of individual plants in different plant development stages) and influence botanical composition (Vibart et al., 2016; Mangwe et al., 2020), and can regulate the maturity of the plants (e.g. amount of older leaf material and dead matter) (Fulkerson and Donaghy, 2001). Therefore, the nutritive value of diverse pastures is highly variable both seasonally and annually, making it difficult to form solid conclusions on changes in quality characteristics, and their relationships with influencing factors (Grace et al., 2019b; Schaub et al., 2020b).

Table 7: Effect of diverse (grass, legume, and herb mixture) and simple (grass and legume mixture) pasture on the crude protein (CP), neutral detergent fibre (NDF), metabolisable energy (ME), and dry matter (DM) parameters.

Source	CP (% DM)		NDF (% DM)		ME (MJ /kg DM)		DM (%)	
	Simple	Diverse	Simple	Diverse	Simple	Diverse	Simple	Diverse
Bell et al. (2020)	-	25.1	-	52.8	-	10.2	-	22.3
Carmona-Flores et al. (2020)	18.3	17.5	41.8	35.9	10.7	10.9	-	-
Totty et al. (2013)	26.2	23.7	33.1	22.8	12.3	12.2	15.8	14.2
Woodward et al. (2013)	19.9	19.3	-	-	11.7	11.8	16.5	10.8
Sanderson (2010)	19.7	20.2	46.4	37.5	-	-	8.0	8.4
Nobilly et al. (2013)	-	-	-	-	12.2	12.0	-	-
Grace et al. (2018)	19.0	19.7	41.1	38.9	-	-	-	-
Edwards et al. (2015)	18.6	18.2	38.1	39.4	11.8	12.0	12.8	14.4
Hearn et al. (2022)	21.4	21.5	33.2	32.4	-	-	-	-
Rodriguez-Firpo et al. (2022)	22.3	23.2	-	-	11.4	11.5	17.8	15.2
Baker et al. (2023a)	17.0.	17.9	44.1	43.1	-	-	19.1	17.1

Diverse pastures are sometimes associated with reduced CP content (see Table 7), and this can be linked to the reduced proportion of legumes in the mixture, compared to simple pastures (Beukes et al., 2014; Romera et al., 2017; Bell et al., 2020; Carmona-Flores et al., 2020). There have also been reported reductions in total N concentration of the whole pasture due to this (Totty et al., 2013; Vogeler et al., 2017). This is not necessarily negative, as the higher total legume content in simple mixtures can result in high CP values and excessive N uptake when animals graze the pasture (Vogeler et al., 2017; Carmona-Flores et al., 2020). This excess N in pastoral systems is associated with N losses to the environment through leaching into groundwater and runoff into surface water, and gaseous losses of N_2O, into the atmosphere (Edwards et al., 2015; Cheng et al., 2018; Carmona-Flores et al., 2020; Cummins et al., 2021).

There is some disagreement between authors as to whether diverse pastures are associated with improved metabolisable energy (ME) or not. This appears to be dependent on the grazing management and botanical composition of the pastures. For example, whilst Schaub et al.

(2020b) and Grace et al. (2019b) reported an increase in ME with increasing diversity of pasture, other authors reported similar or even reduced ME as species diversity increased in pasture. Reports from Woodward et al. (2013), Carmona-Flores et al. (2020), and Totty et al. (2013) showed a similar or non-significant reduction in ME, whilst Nobilly et al. (2013) reported a small reduction over a two-year experiment. It is commonly noted that legumes generally maintain a higher ME content compared to other functional groups (see Appendix 1, Table 16), due to lower fibre content, and greater CP content (Nobilly et al., 2013; Grace et al., 2018; Schaub et al., 2020b). Some studies suggest that the generally higher proportion of legumes, as mentioned earlier, and the generally more frequent and lower grazing management undertaken in simple pastures, could explain this difference in ME (Nobilly et al., 2013; Grace et al., 2018). This is supported by a study by Carmona-Flores et al. (2020), where there was no difference in legume proportion between simple and diverse mixtures, and consequently no difference in ME between the treatments. Whilst Nobilly et al. (2013) reported an average reduction in ME from diverse pastures, they also reported an increased total ME per hectare (ha), which may be due to the increased DM yield of the pasture. The comparatively higher ME in simple pastures could have been partially explained by the consistently low grazing residual reducing the amount of dead matter and stem in the pasture, resulting in a higher ME value of the grass fraction, along with a higher legume content (Nobilly et al., 2013). As discussed earlier, some reports have shown an increase in yield with diverse pastures compared to simple pastures. Golinska et al. (2023) showed that species such as ryegrass reach a certain growth stage slower in mixtures than when in monoculture, and chicory remained in a vegetative stage with reduced reproductive shoots for a longer period compared to monocultures of the species. They concluded that mixtures improve the relationship between DM yield and NV. This is further supported by Schaub et al. (2020a), stating that with increased plant diversity, there was an increase in quality-adjusted yield by increasing yield at a constant forage quality. This could explain the improved ME/ha that Nobilly et al. (2013) found. Therefore, while there have been reported increases in ME of diverse pastures, it is highly dependent on the management, species selection and proportion of functional groups as to whether this advantage is seen.

Diverse pastures are also generally associated with a reduction in NDF (see Table 7), compared to simple pastures (Nobilly et al., 2013; Totty et al., 2013; Pembleton et al., 2015; Grace et al., 2018; Catalano et al., 2020; Jaramillo et al., 2021). However, similar to ME, this is highly dependent on grazing management, botanical composition and age of the pasture (Nobilly et al., 2013; Jing et al., 2017; Grace et al., 2018). This recorded decrease in NDF is reported to be due to the increasing proportion of herbs, especially chicory and plantain (Nobilly et al., 2013;

Grace et al., 2018; Jaramillo et al., 2021). However, in contrast, there are also studies that have reported an increase in NDF in diverse pastures (Deak et al., 2007; Schaub et al., 2020b). This may be explained by the increase in diversity being related to the number of species rather than to functional groups, as these studies had a higher proportion of grass in their diverse pastures compared to simple pastures. Grasses tend to have a higher NDF value when compared to both legumes and herbs (Vibart et al., 2016; Grace et al., 2018). Therefore, compared with simple pastures, diverse pastures containing a lower proportion of grass and a high proportion of herb species in the pasture, would be expected to record lower NDF contents and thus higher ME.

2.6. Pasture management

2.6.1. Simple pastures

Grazing management has a major impact on the production, persistence and quality of pasture, and the two most important aspects of rotational grazing management include grazing interval (or grazing rotation, defined as when a paddock or area should be grazed) and grazing intensity (or post grazing residual, defined as how hard or close to graze a paddock or area) (Donaghy et al., 2021). Some of the most common methods used to determine grazing rotation include rotation length, grazing residual and herbage mass, however, these techniques are based on animal-related indicators, and are driven by feed allocation, area and number of paddocks (Fulkerson and Donaghy, 2001). Furthermore, day rotations, grazing residual height and herbage mass do not take into account climatic variation, pasture density and seasonal variation/plant health condition, respectively. Leaf regrowth stage (or 'leaf stage') is a management tool based on plant indicators (energy reserves and leaf senescence), which has been shown to improve the quality, production and persistence of pasture (Fulkerson and Donaghy, 2001).

There is significant literature on the grazing management of common simple pastures, especially ryegrass and white clover. Grazing rotation is recommended at between the two- and three-leaf stages (Fulkerson and Donaghy, 2001), with a post grazing residual of around 5cm to optimise pasture production, quality and persistence (DairyNZ, 2023). The leaf stage is an effective management tool in simple pastures, as there is a focus on only one species, however, it has not been tested in pastures with multiple species.

2.6.2. Diverse pastures

Compared to simple pastures, there is a dearth of research investigating grazing management of diverse pastures. From a practical management perspective, due to the large number of species that can be included in diverse pastures, and their different characteristics, it is difficult to provide optimal conditions for all species. Furthermore, it depends on the mixture of species as to the management decisions required. Grazing management for diverse pastures may require different techniques compared to simple pastures; for example, due to multiple species within the pasture, any one leaf stage (e.g. based just on ryegrass), is not expected to be the best method for diverse pastures (Donaghy et al., 2021).

Some studies indicate that increased diversity requires an increased focus on management, along with increased inputs, to maintain species diversity (Vibart et al., 2016). For example, some species require specific defoliation dates to maintain long-term persistence (e.g., lucerne) (Pembleton et al., 2015). Also, chicory and plantain are sensitive to defoliation intervals in areas with moisture deficits (Mangwe et al., 2020). The taproot is the main storage organ of water-soluble carbohydrates (WSC), which is used for plant growth after defoliation and survival through winter via dormancy (Mangwe et al., 2020). The level of WSC tends to be lower in more frequently grazed plants, leading to reduced persistence (Li et al., 1997b; Fulkerson and Donaghy, 2001; Li and Kemp, 2005). However, to maintain high quality and production of these herbs, more frequent defoliation (e.g. three-five weeks) is recommended (Hare et al., 1987; Clark et al., 1990; Sanderson et al., 2003; Labreveux et al., 2004; Ayala et al., 2011; Mangwe et al., 2020) along with a minimum grazing residual of 5cm (Li et al., 1994; Li et al., 1997c). Furthermore, it is recommended that plantain is only mildly grazed, or even not grazed, over late-autumn and winter (Li et al., 1997b; Ayala et al., 2011), with Ayala et al. (2011) reporting reduced leaf-to-stem ratio and overall yield in spring, and a 32.2% reduced number of plants in summer, following winter grazing. Legumes, like red clover, require slightly different grazing management to maintain persistence, such as four-six week rotation lengths and a minimum grazing residual height of 8-10cm (Hyslop, 1999; Kemp et al., 1999). This contrasts the minimum residual height of herbs, showing that the optimum grazing preference of one species can have negative effects on other species in the mixture (Cranston et al., 2015b).

Preferential grazing of any one species over a prolonged period leads to that species being unlikely to persist. Selective grazing is likely to reduce the proportion of desirable species (Carmona-Flores et al., 2020), leading to just one or two species becoming dominant over time (Distel et al., 2020). However, if preference changes seasonally, then it can enable species to persist in diverse pastures (Cranston et al., 2015a). Furthermore, there are management techniques that can be used to reduce selective defoliation (Distel et al., 2020).

Information on the management of diverse pastures containing grasses, legumes, and herbs under livestock grazing is limited, especially for those with larger numbers of species. There is evidence to suggest diverse pastures will produce higher annual yields under longer grazing rotation lengths. This has been shown in an experiment by Baker et al. (2023c), where they found a 13% and 18% increase in annual yield when compared to simple pastures under 21- and 28-day rotations, respectively. In the same study, then found that, under both mixtures, increasing rotation length from 21 to 28 days increased annual yield, as there was more time for plants to recover between cutting events, which has been supported in other studies too (Brougham, 1956; Lee et al., 2015; Navarrete Quijada, 2015; Baker et al., 2023c). It is also suggested that increasing the post-grazing residual has a positive impact on the yield of diverse pastures (Clark et al., 1990; Carlassare and Karsten, 2002), and consequently the persistence of herbs (Cranston et al., 2015b). There has also been evidence to show that higher post-grazing residuals favour the growth of red clover and chicory (Cranston et al., 2015b; Baker et al., 2023c), whilst a lower grazing residual height has been shown to favour plantain growth (Cranston et al., 2015b). However, Baker et al. (2023c) found no significant impact on yield with a higher post-grazing residual, although differences between residual heights were smaller than other studies. Some studies suggest that rotation length has a bigger impact on yield than post-grazing residual (Labreveux et al., 2004; Lee et al., 2015; Mangwe et al., 2020).

2.7. Animal production

2.7.1. Milk production

Some studies have shown increased milk yield in cattle with diverse pastures (Totty et al., 2013; Beukes et al., 2014; Edwards et al., 2015; Romera et al., 2017; Distel et al., 2020; McCarthy et al., 2020; Minnée et al., 2020), while others show no significant difference (Woodward et al., 2013; Edwards et al., 2015; Carmona-Flores et al., 2020), but none have shown reductions (Carmona-Flores et al., 2020). Increases in milk production have also been observed in breeding ewes (Hutton et al., 2011; Grace et al., 2019b). The advantage of diverse pastures on milk yield depends on the proportion of certain species in the diet (Carmona-Flores et al., 2020; Distel et al., 2020). For example, increases in milk production have been linked to increased N-use efficiency from the presence of chicory and plantain, especially in the late-lactation diet (Totty et al., 2013; Carmona-Flores et al., 2020; Minnée et al., 2020).

2.7.2. Live weight gain and body condition score

The impact of diverse pastures on livestock performance compared to a simple pasture diet is a subject of significant interest. Findings from multiple studies have highlighted the relationship between the dietary composition of diverse pastures and live weight (LW) gain in both sheep and cattle. Several studies have reported an increase in LW gain for livestock with the inclusion of plantain and/or chicory in their diet (Hutton et al., 2011; Cranston et al., 2015b; Somasiri et al., 2015; Grace et al., 2019b; Beaucarne et al., 2022; Nakajima et al., 2022; Sheridan et al., 2022a). However, this increase is often confined to certain seasons/periods rather than the whole grazing period. (Hutton et al., 2011) reported an increase in ewe LW, specifically during lactation when comparing herb and ryegrass diets. This is also demonstrated in an experiment by Cranston et al. (2015b), where the increased LW gain was more evident in summer (late lactation) when the nutritive value of perennial ryegrass is lower than herbs. Furthermore, studies have found effects of herbs in the diet to have increased LW of both ewes and lambs at weaning, when compared to ryegrass-white clover mixture (Grace et al., 2019b). It is suggested that the increase in LW gain is due to the typically higher quality characteristics of herbs within the diet, particularly over summer when there is a declining quality of perennial ryegrass (Cranston, 2014), allowing for higher feed efficiency from diverse pastures at that period of time (Hutton et al., 2011; Somasiri et al., 2015). There has also been inconsistent and season-dependant results from experiments by Cheng et al. (2018), showing only a small positive difference in the experimental spring period (when the nutritive value of ryegrass is relatively higher than in summer). Therefore, the inclusion of herbs in the diet can have a positive impact on LW gain in livestock, but this is dependent on the season and composition of feed.

Studies have also investigated the relationship between a diverse pasture diet in livestock and changes in body conditions score (BCS) as a performance indicator. Studies have suggested an overall increase in livestock BCS with the addition of herbs in the diet when compared to ryegrass-clover diets (Cranston et al., 2015b; Grace et al., 2019b; Carmona-Flores et al., 2020; Nakajima et al., 2022). There have also been reports of relatively larger BCS from diverse pastures during early-, and mid-to late-lactation, respectively (Hutton et al., 2011; Grace et al., 2019b). There have also been reports of no significant difference in BCS from differences in diet (Corner-Thomas et al., 2014). However, this may have been due to the stage of growth and maturity of younger ewes used in the experiments. Therefore, there is generally a positive relationship between the inclusion of herbs in the diet for grazing livestock and BCS, when compared to ryegrass-clover diets.

2.8. Environmental impacts

Recent research into diverse pastures and environmental impacts explain the multiple pathways to reducing greenhouse gas emission from these mixtures. For example, reductions in methane (CH_4) and N_2O observed, particularly with the presence of chicory and plantain. Non-structural carbohydrates (NSC) are readily fermented in the rumen, which improves the efficiency of nutrient use, and thus less CH_4 is produced (Distel et al., 2020). Urea that is bound with NSC also increases the efficiency of N utilisation in the rumen (Carmona-Flores et al., 2020). Condensed tannins (CT) reduce the degradability of protein in the rumen, reducing the amount of ammonia (NH_3) produced. Plantain and chicory are two examples of pasture species that have higher levels of both CT and NSC compared to other species. Furthermore, both species have a diuretic effect on ruminants, reducing the concentration of N in urine at any one urination event, leading to a reduction in N_2O emissions and N leaching (Vibart et al., 2016). However, the extent of this impact depends on the proportion of plantain in the diet, with some sources claiming that a minimum of 30% is required (Bryant et al., 2019; Minnée et al., 2020), although more recent research disputes this (Agricom, 2024; DairyNZ, 2024).

Another mechanism by which diverse pastures might result in reductions in N leaching and N_2O emissions is that they are often associated with a lower total N concentration partially from reduced legume proportion, and herbs species having lower total N (see Appendix 1, Table 16) (Vibart et al., 2016; Cummins et al., 2021). Furthermore, they often require less N fertiliser to produce the same yield as simple pastures, and this lower N loading on soils will naturally mean less N available to be lost (Cummins et al., 2021).

2.9. Conclusions

Perennial ryegrass and white clover is the most commonly sown pasture mix in NZ. However, seasonal growth patterns, persistence and quality of these pastures can vary. Studies have indicated that quality, production and growth seasonality can be improved with diverse pastures (containing grasses, legumes and herbs), along with improved animal production and reduced environmental impacts. However, there is a dearth of literature on recommended grazing management practices that can maintain species diversity and production in pastoral systems, especially with ‘hyper’ diverse pasture mixtures (more than 9 species). Therefore, the main objective of this thesis is to determine the effects of grazing rotation length and post-grazing residual height on the production, quality and botanical composition of hyper-diverse pastures.

CHAPTER 3. MATERIALS AND METHODS

3.1. Plant establishment

A field experiment was set up on the Massey University Pasture and Crop Research Unit, located on Poultry Farm Road, 5 km south of Palmerston North, NZ (-40.38177, 175.60813). The soil type was a Manawatu fine sandy loam (Dystric Fluventric Eutrochrept) (Hewitt, 1988). The climate in this area is warm-temperate (see Table 8) with an annual rainfall of 1839 mm and mean maximum and minimum daily air temperatures of 20.2 ℃ and 9.2 ℃, respectively. The experimental site was irrigated on 26 January 2023 (20 mm applied) when herbage growth was severely limited due to lower than average monthly rainfall (see Appendix 2, Figure 10).

The study site was prepared by spraying with Weedmaster 360 (360g/L of glyphosate) at 3L/ha, followed by ploughing and secondary cultivation. The site was split up into 96, one by three metre plots that included two pasture mixes (‘sheep’ and ‘dairy’ diverse pasture mixtures, see later for description (see Table 9). Plots were sown in a randomised block design on 5 May 2022 with an Oyjord plot drill, followed by the whole area being rolled. All plots were mowed on the 10^{th} of October 2022 (week zero) to 63.5 mm with a flex-wing rotary mower. This was undertaken to ensure consistent pasture residual height across plots and to reduce competition from shading of neighbouring plots. All further defoliation was undertaken by a rotary mower with a rear catcher.

Table 8: Mean monthly soil moisture deficit, maximum and minimum air temperatures (°C), total monthly rainfall and irrigation water applied (mm) between October 2022 and May 2023 compared with the 10-year mean. Measurements were collected from a weather station.

	Oct.	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May.**	**Average**
Experiment (October 2022 to May 2023)									
Max. daily air temperature (°C)	16.98	20.33	23.19	26.54	24.56	19.97	18.13	15.55	20.66
Min. daily air temperature (°C)	6.36	9.36	11.33	13.74	11.95	8.69	8.69	7.42	9.69
Rainfall (mm)	99.40	215.60	95.60	48.00	51.40	176.60	209.40	246.80	142.85
Soil moisture deficit (mm)	10.36	7.92	18.16	83.08 (20)*	105.35	52.77	1.75	0.58	35.00
Mean (10 year)									
Max. daily air temperature (°C)	17.14	20.07	22.25	23.89	24.16	22.30	17.84	14.19	20.23
Min. daily air temperature (°C)	6.84	8.86	10.93	11.82	11.65	10.38	7.60	5.35	9.18
Rainfall (mm)	151.26	141.48	131.48	121.66	114.52	98.54	155.44	201.46	139.48
Soil moisture deficit (mm)	5.85	19.37	31.80	52.52	62.62	62.91	23.00	1.43	32.44

*Irrigation applied (m)

Table 9: Individual species in the dairy and sheep pasture mix plots and their sowing rates.

Pasture Type	Species	Equivalent Sowing Rate (kg/ha)
I) Dairy	*Lolium perenne* L. (cv. Platform AR37)	4.6
	Lolium perenne L. (cv. 4 Front)	4.0
	Festuca arundinacea (Schreb.) (cv. Quantica MaxP)	4.0
	Festuca pratensis Huds. (cv. Oakdon MaxR)	4.0
	Dactylis glomerata L. (cv. Savvy)	1.9
	Phleum pratense L. (cv. Kahu)	1.9
	Phalaris aquatica L.	1.0
	Bromus catharticus Vahl. (cv. Atom)	4.0
	Trifolium pratense L. (cv. Relish)	4.0
	Trifolium repens L. (cv. Kotuku)	1.9
	Trifolium repens L. (cv. Weka)	2.0
	Cichorium intybus L. (cv. Choice)	1.1
	Plantago lanceolata L. (cv. Ecotain)	1.9
	Trifolium michelianum Savi. (cv. Viper)	1.9
	Trifolium resupinatum L. (cv. Laser)	1.9
	Trifolium incarnatum L.	3.0
	Vicia villosa Roth.	2.0
	Medicago sativa L.	4.0
II) Sheep	*Lolium perenne* L. (cv. Maxsyn NEA4)	4.0
	Lolium perenne L. (cv. 4 Front)	4.0
	Festuca pratensis Huds. (cv. Oakdon MaxR)	4.0
	Dactylis glomerata L. (cv. Savvy)	2.0
	Phleum pratense L. (cv. Kahu)	2.0
	Trifolium pratense L. (cv. Relish)	4.0
	Trifolium repens L. (cv. Kotuku)	2.8
	Trifolium repens L. (cv. Weka)	1.4
	Cichorium intybus L. (cv. Choice)	1.4
	Plantago lanceolata L. (cv. Ecotain)	2.0
	Lotus corniculatus L.	2.0
	Onobrychis viciifolia Scop.	2.0
	Sanguisorba minor Scop.	1.4
	Trifolium michelianum Savi. (cv. Viper)	2.0
	Trifolium resupinatum L. (cv. Laser)	2.0
	Trifolium vesiculosum Savi. (cv. Zulu II)	2.0
	Trifolium subterraneum L. (cv. Coolamon)	2.0
	Trifolium fragiferum L.	2.0
	Vicia villosal Roth.	2.0

3.2. Experimental design

Defoliation residual height (DH) and interval or frequency (DF) treatments were randomly allocated to plots after sowing. The treatments included four DHs; 25.4 mm (A), 50.8 mm (B), 76.2 mm (C) and 101.6 mm (D) and three DFs; three weekly (3), six weekly (6) and nine weekly (9). A randomised block design of 24 treatments (two pasture mixes, four DHs and three DFs; 24 treatments) was utilised (see Figure 1). The randomised block design was replicated four times, resulting in 96 individual plots. On the 8th of November 2022 (Week 0), all plots were cut to 63.5mm. The treatments began after this date and were continued for the following 27 weeks (refer to Table 3 for exact timings and dates of cuts and samples collected). This resulted in nine, six, and three sampling dates for three, six, and nine-weekly DFs, respectively.

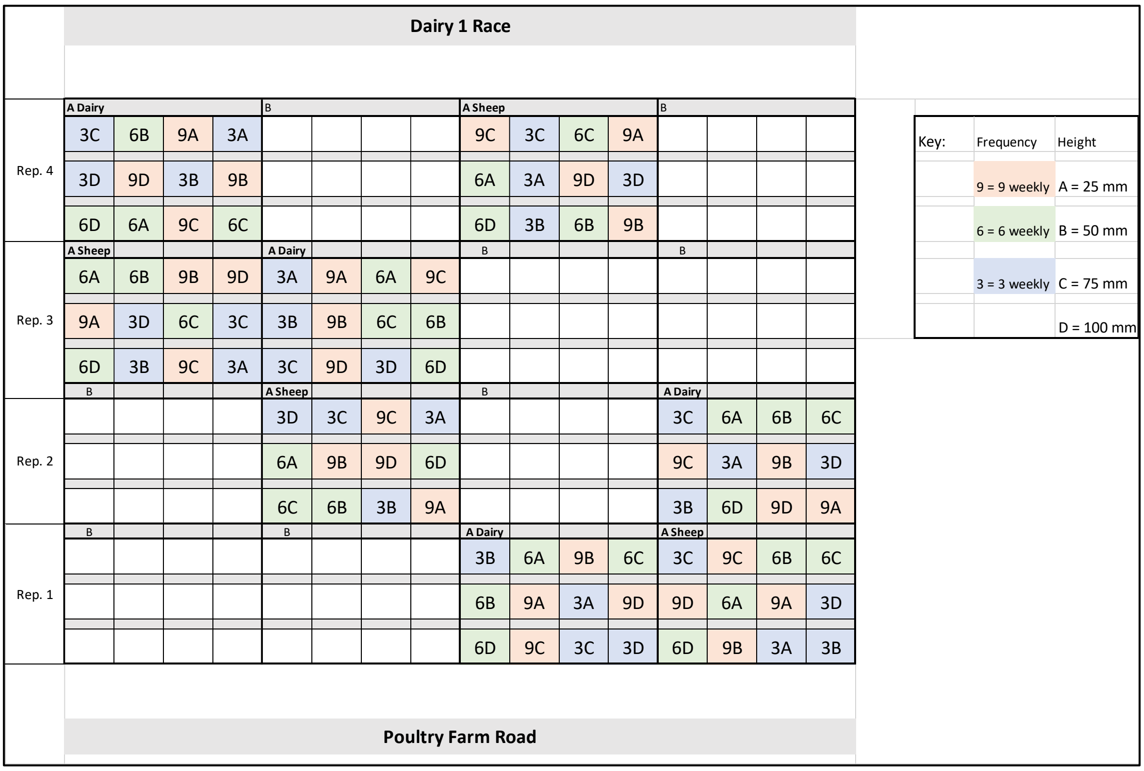

Figure 1: Experimental layout.

3.3. Herbage cut measurements

Herbage mass and growth rate (kg DM/ day) were measured by mowing, weighing, and drying samples from individual plots at their appropriate residual heights and frequencies (see Appendix 3 Table 17). Samples collected were oven-dried at 60°C to a constant weight (over approximately 72 hours), to estimate dry matter (DM) yield.

Residual cuts were taken from all three and nine-weekly DF treatments on the 16th May 2023, and from the six-weekly DF treatment on the 6th June 2023 to ground level using the same technique as samples collected for botanical composition (see botanical measurements) after mowing to the respective residual height.

3.4. Plate meter measurements

Growth rate (kg DM/day) was measured also using a rising plate meter (Jenquip EC20, Fielding, New Zealand) with 30 measurements per plot taken every three weeks, with an additional measurement immediately post-harvest for plots harvested. Measurements were taken by walking a zig-zag pattern across the small plots with the following equation; $\text{Pasture Production} = \text{compressed sward height (H)} \times 140 + 500$.

3.5. Botanical composition measurements

Herbage subsamples (approximately 100g wet weight) were collected for botanical composition analysis from each replicate at each respective cutting event. Botanical composition was measured by cutting three strips (300mm x 77mm) in random positions in each individual plot, to its allocated treatment residual height before mowing the remaining plot area. Botanical samples were sorted into grasses (including those not sown), forbs [plantain, chicory, and sheep's burnet (*Sanguisorba minor*)], legumes (Fabaceae family), dead matter (more than 50% of the sample with necrosis) and others (broad-leaved weeds and other species not sown). These samples were oven-dried at 60^{o}C for approximately 72 hours to estimate DM yield and percentage of each species within each treatment replicate.

Visual (non-destructive) botanical composition was measured in-field using a 0.09m^2 quadrat with 36 cells (50 x 50mm) placed in the centre of each plot. The absence and presence of each sown species in individual cells was recorded in all 96 plots on 13th December 2022 (Week 5), 28th February 2023 (Week 16), and 9th May 2023 (Week 26). See Appendix 4, Tables 18, 19, and 20 for identification resources used.

3.6. Herbage quality measurements

Herbage quality was assessed using the cut herbage samples dried for herbage mass and growth rate measurements. Post-drying, samples were ground through a 1 mm sieve before being sent to Hills Laboratory (R J HILL LABORATORIES LIMITED, Hamilton, New Zealand) for wet chemistry analysis. Only three and nine-weekly DF treatments were analysed from samples collected on 16th May (Week 27).

Method validation for quality analysis was completed from nine-weekly DF samples at DH of H2 and H3. Three subsamples from each replicate of these treatments were collected on 16th May, with each treatment being dried at 60, 95, and -35°C and subsequently ground through a 1 mm sieve before being sent to Hills Laboratory for wet chemistry analysis (see Appendix 6 Table 20).

3.7. Statistical analysis

All data were analysed using RStudio (R Version 4.2.0, R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics generated the following data using the means function including standard errors of the mean (SE).

Pre- and post-cutting herbage mass and apparent DM removal data were analysed using a linear regression model (lm model in package stats 3.6.2). Two- and three-way interactions were tested and were not statistically significant so were removed from further analysis. All models included pasture mixture (dairy or sheep), cutting interval (three, six, or nine weeks) and cutting residual height (25, 50, 75, and 100mm) were included as fixed effects and block was fitted as a random effect. Season was also included as fixed effect, where the models output corresponded to the seasonal production for the analyses of pasture yield (kg DM/ha).

Total apparent DM removal and net herbage accumulation data were analysed using a linear regression model. Two- and three-way interactions were tested and were not statistically significant so were removed from further analysis. All models included pasture mixture (dairy or sheep), cutting interval (three, six, or nine weeks) and cutting residual height (25, 50, 75, and 100mm) were included as fixed effects and block was fitted as a random effect.

Botanical composition data were plotted using boxplots (ggplot2 boxplot) and were visually assessed for trends in the percentage of functional groups and species separately.

Pasture quality data were analysed using a linear regression model. Two- and three-way interactions were tested and were not statistically significant so were removed from further analysis. All models included pasture mixture (dairy or sheep), cutting interval (three, six, or nine weeks) and cutting residual height (25, 50, 75, and 100mm) were included as fixed effects and block was fitted as a random effect.

CHAPTER 4. RESULTS

4.1. Apparent dry matter removal

Least squares means and SE for the main effects of treatment and season on pre-, post-cutting dry matter (DM) (kg DM/ha), and apparent DM removal (kg DM/ha) parameters during summer are presented in Table 10. There were no significant differences between the effects of seed mixtures. However, there was a trend for the sheep mixture to generally have greater pre- and post-cutting herbage mass and greater apparent DM removal, except for treatments D3-75, D3-100 and D9-75. There were significant differences between the effects of cutting residual height for 14 out of 24 treatments for the pre-cutting herbage mass (see Table 10). There was a trend where a higher residual height generally had positive effects on all data, except for treatments D9-50, D9-100 and S9-100. There were significant differences between pre-cutting herbage masses at different cutting frequencies. There were also significant differences in apparent DM removal at cutting frequencies (except for D6-75). However, there were significant differences between post-cutting herbage masses by cutting interval. There was a trend whereby longer cutting interval generally had positive effects on all data, except for treatment S9-50.

Table 10: Pre- and post- cut herbage mass and apparent dry matter (DM) removal during summer from rising plate meter data.

Mix	Treatment		Pre-cut DM (kg DM/ha)	Post-cut DM (kg DM/ha)	Apparent DM removal (kg DM/ha)
	Frequency (weeks)	**Height (mm)**			
Dairy	3	25	1641 ± 128.1	1063 ± 47.7	1352 ± 139.0
		50	2276 ± 181.3	1637 ± 67.4	1957 ± 196.6
		75	2779 ± 181.3	2209 ± 67.4	2494 ± 196.6
		100	3207 ± 181.3	2657 ± 67.4	2932 ± 196.6
	6	25	2681 ± 239.8	1159 ± 89.2	1962 ± 240.8
		50	3223 ± 239.8	1832 ± 89.2	2586 ± 240.8
		75	3226[1] ± 239.8	2230 ± 89.2	2840[1] ± 240.8
		100	3917 ± 239.8	2720 ± 89.2	3447 ± 240.8
	9	25	4629[1] ± 314.0	1202 ± 116.8	2796 ± 260.1
		50	4439 ± 314.0	1864 ± 116.8	3149[1] ± 260.1
		75	5128 ± 314.0	2464 ± 116.8	3939 ± 260.1
		100	4716[1] ± 314.0	2863 ± 116.8	3977[1] ± 260.1
Sheep	3	25	1841 ± 181.3	1149 ± 67.4	1495 ± 196.6
		50	2321 ± 181.3	1706 ± 67.4	2013 ± 196.6
		75	2585[1] ± 181.3	2136 ± 67.4	2360[1] ± 196.6
		100	3084 ± 181.3	2562 ± 67.4	2823 ± 196.6
	6	25	3122 ± 239.8	1189 ± 89.2	2275 ± 240.8
		50	3600[1] ± 239.8	1813 ± 89.2	2783 ± 240.8
		75	3616[1] ± 239.8	2265 ± 89.2	3048[1] ± 240.8
		100	3930[1] ± 239.8	2625 ± 89.2	3501[1] ± 240.8
	9	25	4632[1] ± 314	1242 ± 116.8	2891 ± 260.1
		50	4786[1] ± 314	1806 ± 116.8	3482 ± 260.1
		75	4701[1] ± 314	2365 ± 116.8	3584[1] ± 260.1
		100	5349 ± 314	2777 ± 116.8	4409 ± 260.1

[1]NS difference between effects of height on data within mixtures.

Least squares means and SE for the effects of treatment and season on pre- post-cutting DM, and apparent DM removal (kg DM/ha) parameters during autumn are presented in Table 11. There were no significant differences between the effects of mixtures on any treatments or interactions. However, there was a trend for the sheep mixture to have a larger pre- and post-cutting herbage mass and apparent DM removal, except for post-cutting treatments S9-25 and S9-75, and all treatments except for S3-75, S3-100 and S9-75. There were no significant differences between the effects of cutting residual height on any result, except for the apparent DM removal in treatment S3-100. There was a slight trend for higher residual heights to have positive effect on all measurements. There were no significant differences between the effect of cutting interval on treatments, except for 12 out of the 96 treatments across all measurements (see Table 11). There was a trend where longer cutting interval had a positive effect on all treatments, except for pre-cutting herbage mass in treatments D6-75, D6-100, S6-100, and post-cutting herbage mass at nine-week cutting frequencies.

Table 11: Pre- and post- cutting herbage mass and apparent dry matter (DM) removal during autumn from rising plate meter data.

Mix	Treatment Frequency (weeks)	Height (mm)	Pre-cut DM (kg DM/ha)	Post-cut DM (kg DM/ha)	Apparent DM removal (kg DM/ha)
Dairy	3	25	1011 ± 246.6	1002 ± 257.9	1013 ± 175.6
		50	1364 ± 348.8	1329 ± 364.7	1359 ± 248.3
		75	1782 ± 348.8	1733 ± 364.7	1762 ± 248.3
		100	2248 ± 348.8	2202 ± 364.7	2224 ± 248.3
	6	25	1099 ± 402.7	1711[1] ± 341.1	1426 ± 256.0
		50	1481 ± 402.7	2273[1] ± 341.1	1908[1] ± 256.0
		75	1743[a] ± 402.7	2573[1] ± 341.1	2198 ± 256.0
		100	2135[a] ± 402.7	3056[1] ± 341.1	2662 ± 256.0
	9	25	2817[1] ± 402.7	1096 ± 364.7	1864 ± 265.5
		50	3041[1] ± 402.7	1482 ± 364.7	2162 ± 265.5
		75	3502[1] ± 402.7	1835 ± 364.7	2588 ± 265.5
		100	3647[1] ± 402.7	2082 ± 364.7	2774 ± 265.5
Sheep	3	25	1094 ± 348.8	1035 ± 364.7	1077 ± 248.3
		50	1466 ± 348.8	1401 ± 364.7	1452 ± 248.3
		75	1753 ± 348.8	1629 ± 364.7	1707 ± 248.3
		100	2227 ± 348.8	2194 ± 364.7	2219 ± 248.3
	6	25	1133 ± 402.7	2014[1] ± 341.1	1621[1] ± 256.0
		50	1499[a] ± 402.7	2475[1] ± 341.1	2023[1] ± 256.0
		75	1765[a] ± 402.7	2738[1] ± 341.1	2309[1] ± 256.0
		100	2140 ± 402.7	3123[1] ± 341.1	2708 ± 256.0
	9	25	2870[1] ± 402.7	1089[a] ± 364.7	1889 ± 265.5
		50	3455[1] ± 402.7	1462[a] ± 364.7	2355 ± 265.5
		75	3226[1] ± 402.7	1723[a] ± 364.7	2388 ± 265.5
		100	3716[1] ± 402.7	2183[a] ± 364.7	2903 ± 265.5

[1]Significant difference between the effects of frequency on data

[a]Negative relationship between the effects of frequency on data

Least squares means and SE for the effects of treatment on total apparent DM removal (kg DM/ha) parameters are presented in Table 12. There were no significant differences between mixtures on any treatment combination. However, there was a trend for the sheep mixture to have a positive effect on all results, except for treatments S3-75, S3-100, and S9-100. There was a significant difference between the effect of cutting residual height in 18 out of 24 treatments (see Table 12). There was a trend where higher cutting residual heights having a positive effect on all results. There was a significant difference between the effect of cutting interval in 20 out of 24 treatments (see Table 12). There was a trend for longer cutting interval to have a positive effect on all results.

Table 12 : Total apparent dry matter (DM) removal from rising plate meter data.

Mixture	Treatment Frequency (weeks)	Height (mm)	Total apparent DM removal (kg DM/ha)
Dairy	3	25	1182 ± 116
		50	1655 ± 164
		75	2118 ± 164
		100	2561 ± 164
	6	25	1632 ± 185
		50	2169 ± 185
		75	2445[1,2] ± 185
		100	2964 ± 185
	9	25	2203 ± 195
		50	2521[1,2] ± 195
		75	3079[2] ± 195
		100	3211[1,2] ± 195
Sheep	3	25	1282 ± 164
		50	1727 ± 164
		75	2026[1] ± 164
		100	2504 ± 164
	6	25	1872 ± 185
		50	2316 ± 185
		75	2593[1] ± 185
		100	3013 ± 185
	9	25	2254 ± 195
		50	2765 ± 195
		75	2823[1] ± 195
		100	3451 ± 195

[1]NS difference between effects of cutting height on data
[2]NS difference between effects of cutting frequency on data

4.2. Herbage accumulation

Least squares means and SE for the effects of treatment on harvested herbage mass (kg DM/ha) and residual herbage mass (kg DM/ha) parameters and least squares means of total herbage mass accumulation are presented in Table 13. There were no significant differences between the effect of mixtures on any treatments, except for D9-50. There were no significant differences between the effect of residual height on any treatments, except for 7 out of the 48 treatments across harvested herbage mass and residual herbage mass (Table 13). There were no significant differences between the effect of interval on any treatments, except for 2 out of the 48 treatments across harvested herbage mass and residual herbage mass (see Table 13). However, there was a trend for the sheep mixture to have a positive effect on harvested herbage mass for 19 out of 24 treatments (Table 13) and a trend for the dairy mixture to have a positive effect on residual and total herbage mass except for D3-25, D6-50, D6-100, D9-50, D9-75, and D9-100. There was a trend for higher cutting residual height to have a negative effect on harvested herbage mass, except for treatment D3-75. There was also a trend for higher cutting residual height to have a positive effect on residual herbage mass, except for treatment D6-75, and total herbage mass accumulation, except for treatments D6-50, D6-100, D9-25-100, S6-75, and S9-75. There was a trend for longer interval to have a positive effect on harvested herbage mass and total herbage mass accumulation. There was no clear trend for interval and residual herbage mass.

Table 13 : Net herbage accumulation (kg DM/ha) from cutting data.

Mixture	Frequency (weeks)	Height (mm)	Harvested herbage mass (kg DM/ha)	Residual herbage mass (kg DM/ha)	Total herbage mass accumulation (kg DM/ha)
Dairy	3	25	809 ± 142	319[2] ± 256.5	1129
		50	752 ± 201	922 ± 362.7	1674
		75	792[1] ± 201	1100 ± 362.7	1893
		100	628[1] ± 201	1538 ± 362.7	2166
	6	25	2211 ± 257	615 ± 362.7	2826
		50	2022 ± 257	685 ± 362.7	2708
		75	1702 ± 257	1845 ± 362.7	3548
		100	1613 ± 257	993[2,3] ± 362.7	2607
	9	25	3683[1] ± 285	697 ± 362.7	4381
		50	2912 ± 285	867 ± 362.7	3779
		75	2636[1] ± 285	1112 ± 362.7	3748
		100	1885[2,3] ± 285	1439 ± 362.7	3325
Sheep	3	25	911 ± 201	229 ± 362.7	1141
		50	812 ± 201	639 ± 362.7	1452
		75	652 ± 201	1082 ± 362.7	1734
		100	608 ± 201	1249 ± 362.7	1857
	6	25	2408 ± 257	314 ± 362.7	2722
		50	2315 ± 257	662 ± 362.7	2977
		75	1845 ± 257	809 ± 362.7	2655
		100	1649 ± 257	1572[2] ± 362.7	3221
	9	25	3641 ± 285	231 ± 362.7	3872
		50	3573 ± 285	673 ± 362.7	4246
		75	2455[2] ± 285	1267[2] ± 362.7	3723
		100	2400 ± 285	2184[2,3] ± 362.7	4584

[1]Significant difference between the effects of mixture on data
[2]Signficant difference between effects of height on data
[3]Significant difference between effects of frequency on data

4.3. Botanical composition

4.3.1. Destructive botanical composition

Mean, interquartile and 95% confidence interval for botanical composition (functional groupings) are presented as boxplots in Figure 2. There is a significant difference in the percentage of legume and herbs in the pasture compared to other groups. Where herbs and legume made up a significantly greater mean percentage of the pasture. Generally, herbs had the greatest mean percentage out of all functional groups, followed by legumes and then grasses. Legumes had the greatest spread of data (0-95% of pasture composition) compared to all other functional groups.

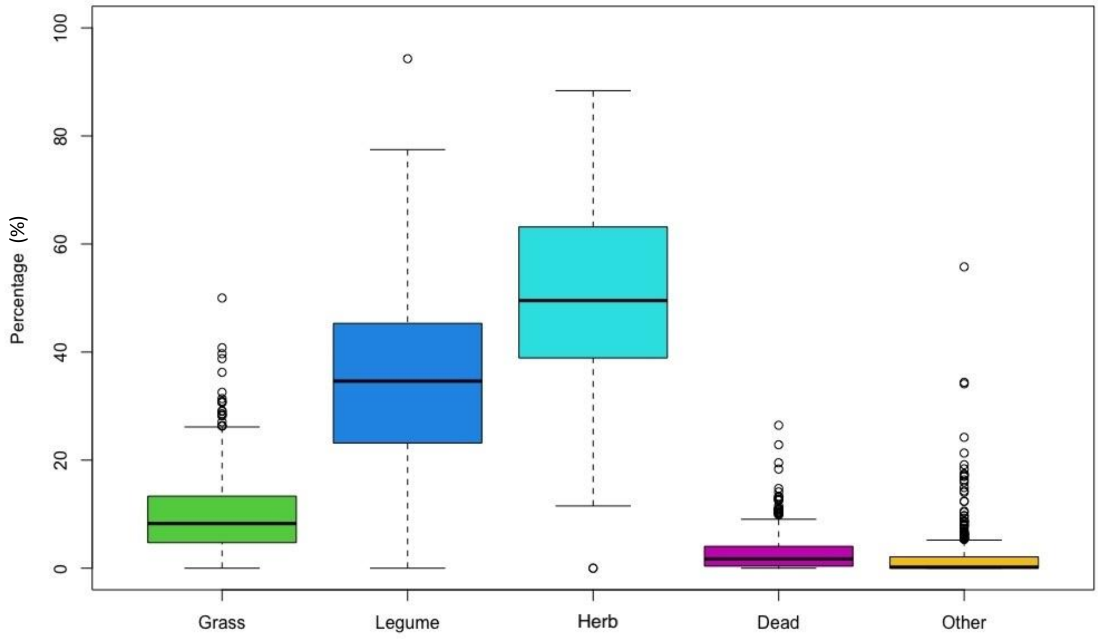

Figure 2: Botanical composition (percentage of grass, legume, herb, dead matter and other) of diverse pastures under all post-cutting residual heights (25mm, 50mm, 75mm, and 100mm).

Mean, interquartile and 95% confidence intervals for botanical composition (functional groupings) for each cutting residual height treatment are presented in Figure 3. There were no significant differences in the mean percentage of herb or legume at any cutting residual height. However, there was a trend for increasing cutting residual height to have a positive effect on legume percentage and a negative effect on herb percentage. There was also a trend where increasing cutting residual height resulted in smaller and greater range percentages for grasses and legumes, respectively. Overall, herbs had the greatest mean percentage and legumes had the greatest spread of data.

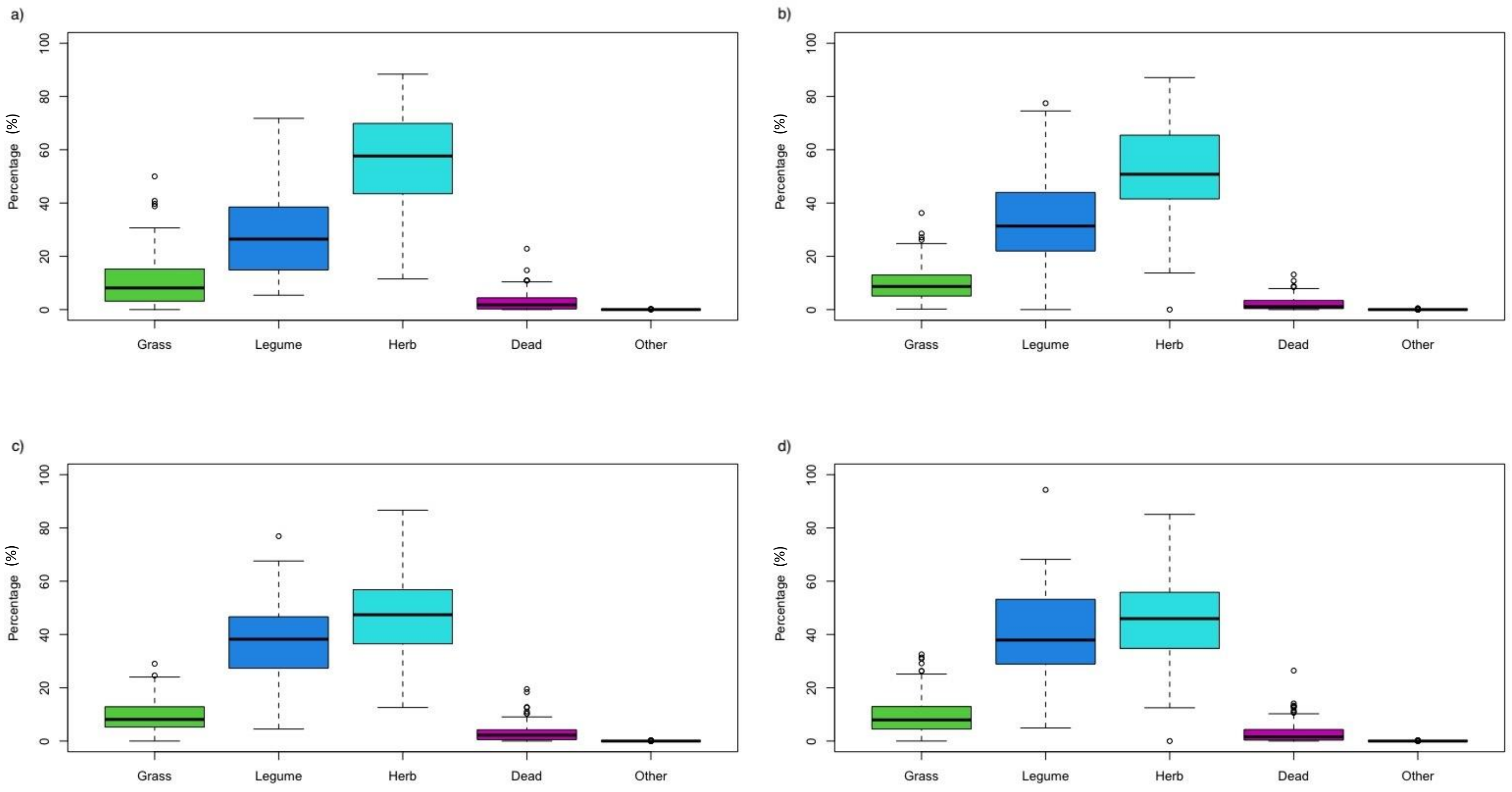

Figure 3: Botanical composition (percentage of grass, legume, herb, dead matter and other) of diverse pastures under the following post-cutting residual heights; a) 25mm, b) 50mm, c) 75mm, d) 100mm.

Mean, interquartile and 95% confidence interval of botanical composition (functional groupings) by cutting interval treatment are presented in Figure 4. There were no significant differences in the mean percentage of herbs vs legumes. However, there was a trend where longer cutting interval increased the percentage of legume and dead matter in the pasture while decreasing the percentage of grasses and herbs. There was a trend where longer cutting interval resulted in a reduced range of observed percentage of the pasture composed by grass, legume, and herb grouping, and a greater range of data for the "other" group (weeds and non-planted species). Overall, herbs had the greatest mean percentage, except for the nine-week treatments, where legume was the greatest. The herb group also had the greatest spread of data.

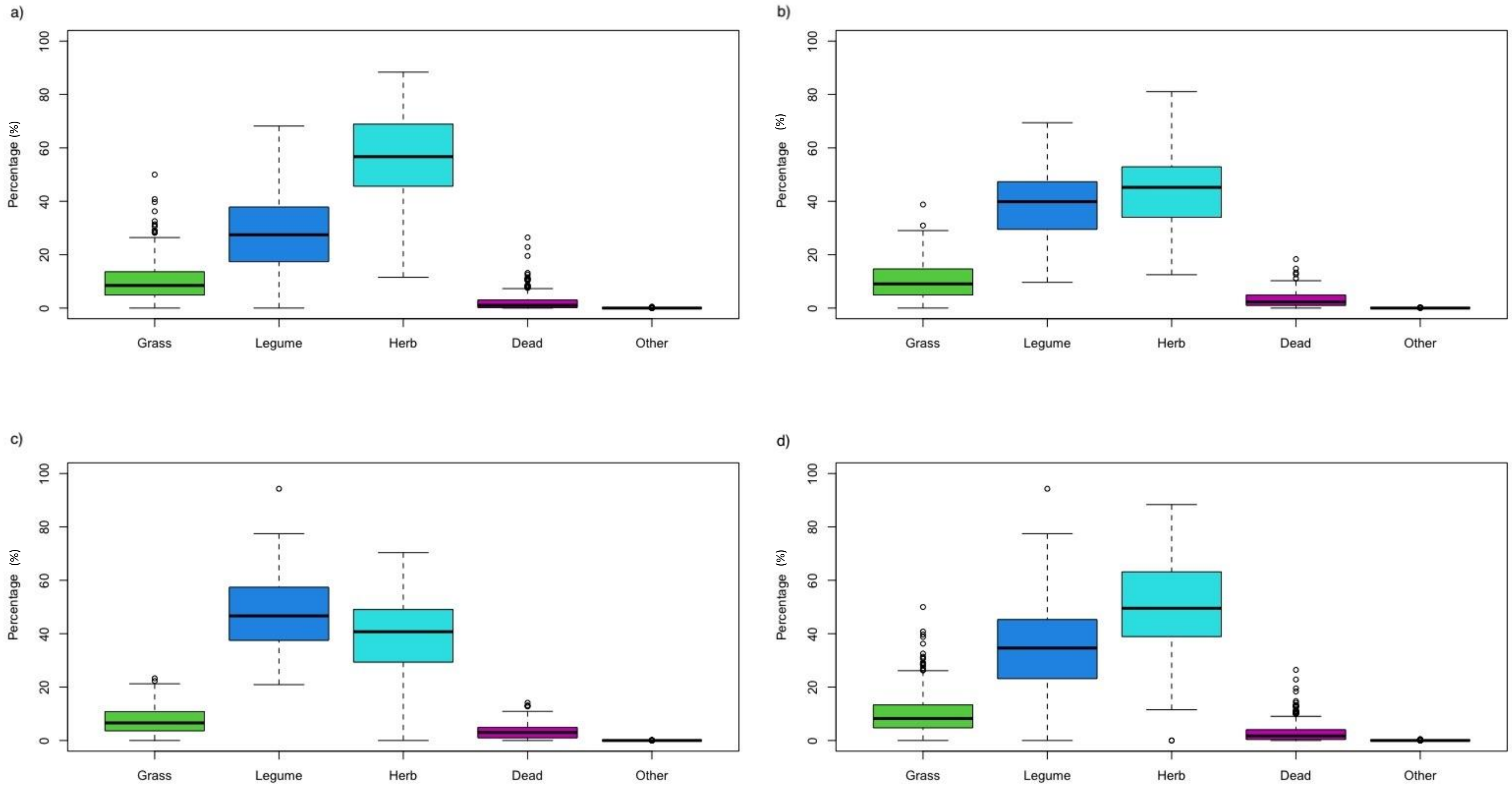

Figure 4: Botanical composition (percentage of grass, legume, herb, dead matter, and other) of diverse pastures under the following cutting frequencies; a) all, b) 3 weeks, c) 6 weeks, d) 9 weeks.

4.3.2. Visual botanical composition

Throughout the experiment, the following were not identified in any plots at any point of the study; Phalaris (*Phalaris aquatica* L.), sanfoin (*Onobrychis viciifolia* Scop.), lucerne (*Medicago sativa* L.), strawberry clover (*Trifolium fragiferum* L.) and hairy vetch (*Vicia villosa* Roth.). The following common weeds were identified throughout the experiment, and presence was scored; summer grass [*Digitaria ciliaris* (Retz.)] and broad-leaved dock (*Rumex obtusifolius* L.). Out of the 18 sown species in the dairy mixture and the 19 sown species in the sheep mixture (Table 9), dominant species throughout the experiment were identified as; plantain (*Plantago lanceolata* L.), red clover (*Trifolium pratense* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). To a lesser extent, chicory and cocksfoot had a small contribution to dominant species with a minor component of birdsfoot trefoil (*Lotus corniculatus* L.) and meadow fescue (*Festuca pratensis* Huds.).

Mean, interquartile and 95% confidence interval of botanical composition (key species) across all treatments are presented in Figure 5. Overall, white clover had the greatest mean percentage and red clover had the greatest spread of data.

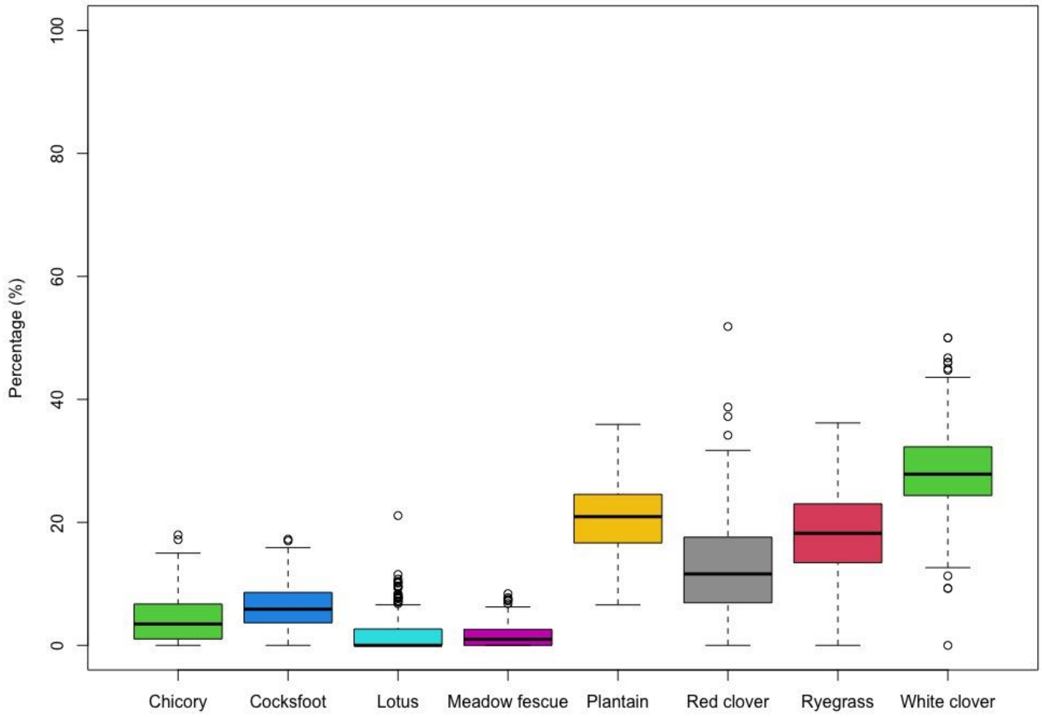

Figure 5: Botanical composition (percentage of the top eight most dominant species) of diverse pastures under all post-cutting residual heights (25-100mm).

Mean, interquartile and 95% confidence interval of botanical composition (key species) at different cutting residual heights are presented in Figure 6. There were no significant differences in the mean percentage of any species between cutting residual height treatments. Between species, white clover had the greatest mean percentage and red clover had the greatest spread of data. There was a trend for increasing cutting residual height to have a positive effect on the mean plantain percentage within a pasture, with the exception of treatment H3. There was also a trend for increasing cutting residual height to result in a reduction in the spread of data for red and white clovers and a greater spread of data for perennial ryegrass.

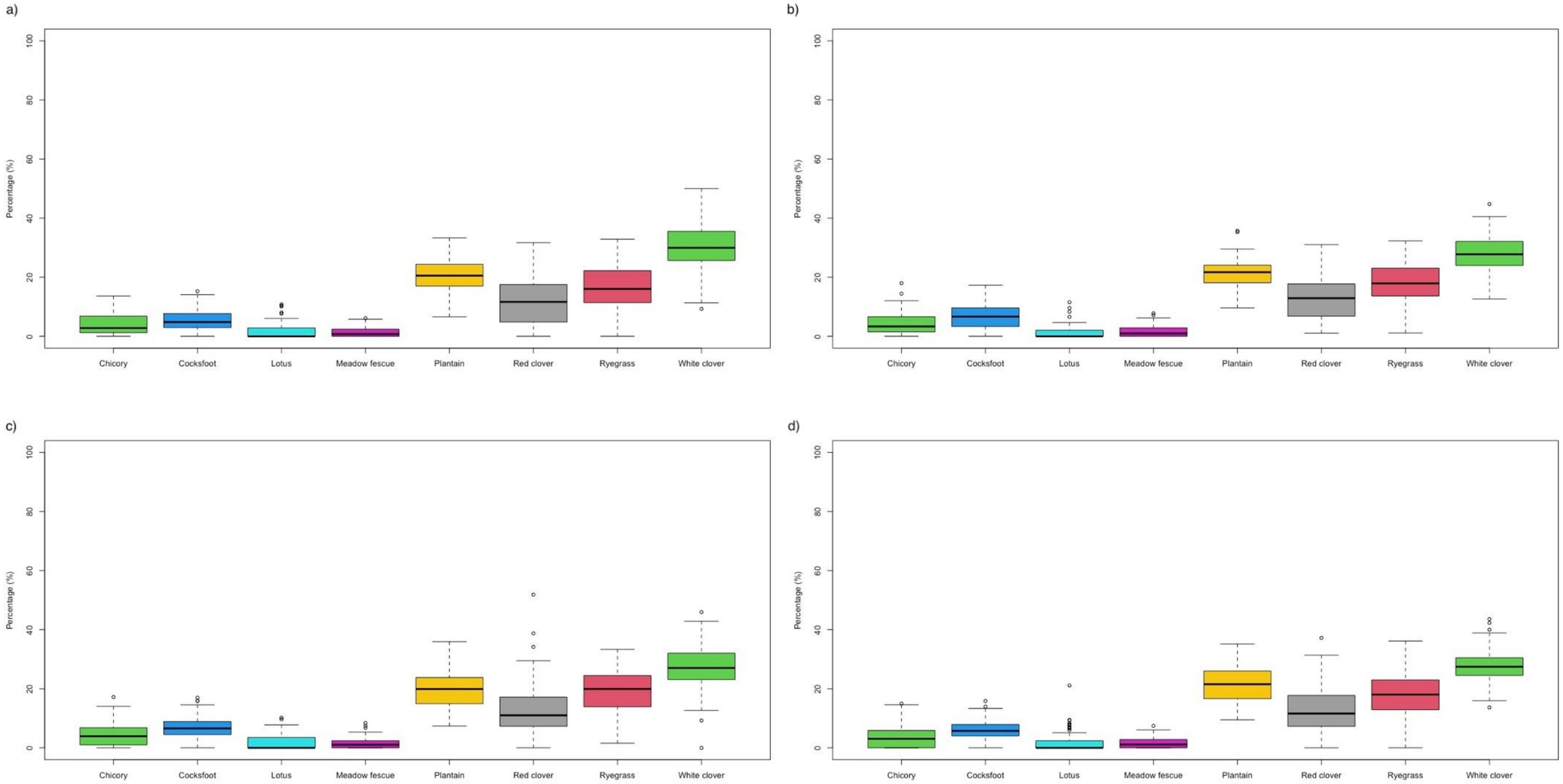

Figure 6: Botanical composition (percentage of the top eight most dominant species) of diverse pastures under the following post-cutting residual heights; a) 25mm, b) 50mm, c) 75mm, d) 100mm.

Mean, interquartile and 95% confidence interval of botanical composition (key species) across cutting frequencies are presented in Figure 7. Overall, white clover had the greatest mean percentage of all key species, and red clover had the largest variation in the percentage of the pasture (except for DF 6 weeks where perennial ryegrass had the greatest spread). There were no significant differences between mean percentages of species by cutting interval. However, there was a trend for longer cutting intervals to result in a wider variation of measurements for red and white clovers. There was also a trend for longer cutting intervals to result in reduced mean percentage of perennial ryegrass and white clover in the pasture and an increased mean percentage of red clover.

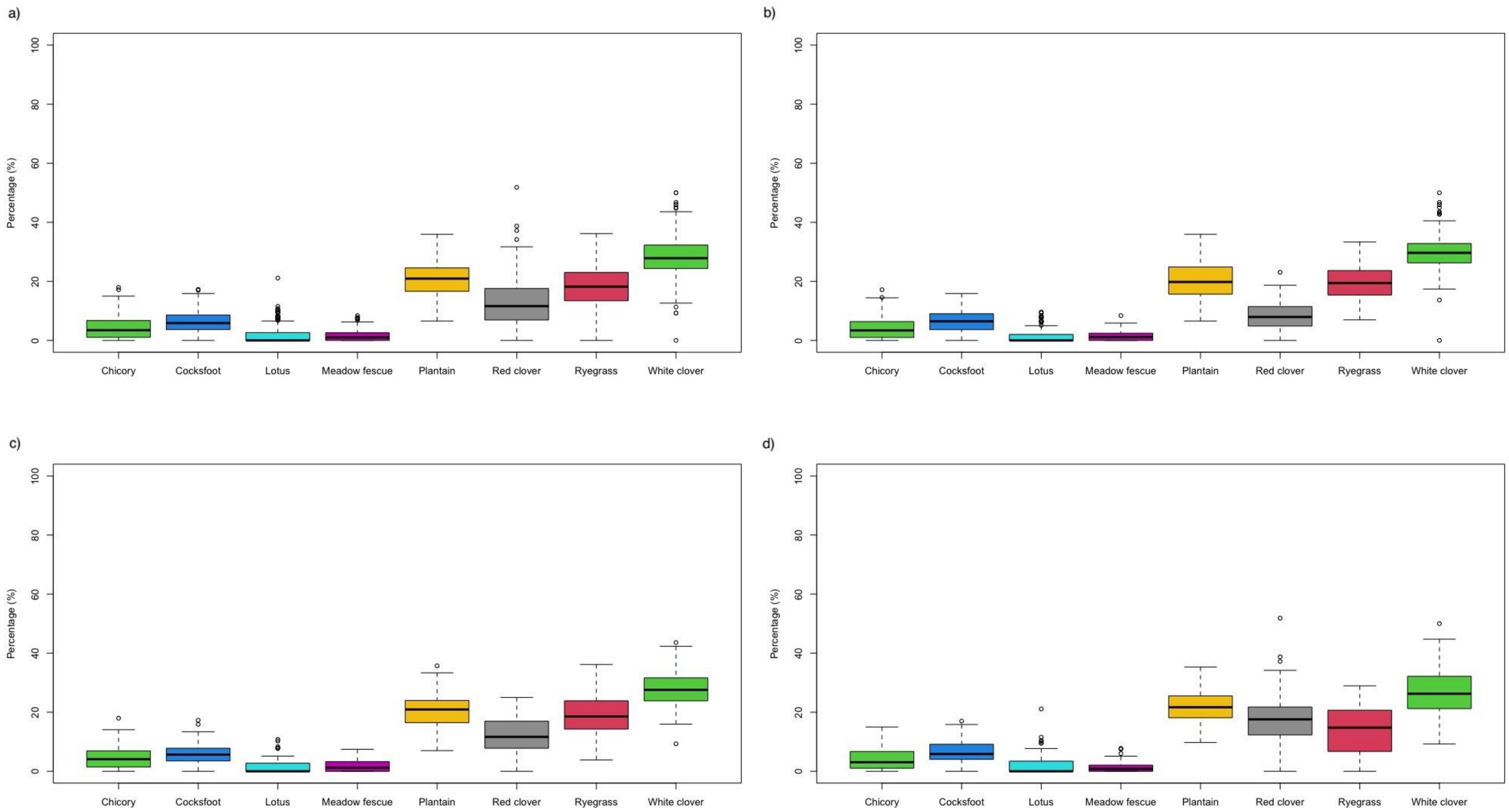

Figure 7: Botanical composition (percentage of the top eight most dominant species) of diverse pastures under the following cutting frequencies; a) all, b) 3 weeks, c) 6 weeks, d) 9 weeks.

4.4. Quality

Least squares means and SE for the effect of treatment on in-vitro organic matter digestibility (OMD), nitrogen (N), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin, organic matter (OM), and metabolisable energy (ME) parameters are presented in Table 14. There were no significant differences between the effects of mixtures on any variables, except for S9-50. There was a trend for OMD to be greater in dairy mixtures and under three-week cutting interval treatments (except for S3-75). Nitrogen content was greater under nine-week cutting interval treatments (except for S9-50 and S9-25). Crude protein was greater in the sheep mixture, except for S9-25 and S9-100. Neutral detergent fibre was greater in the sheep mixture, except for D9-50 and D9-75. Longer cutting intervals resulted in greater NDF content. Lignin content was greater under nine-week cutting interval treatments. Metabolisable energy was greater in the dairy mixture, except for D9-50. There was also a trend for three-week cutting interval treatments to have a greater ME.

Table 14 : Effect of cutting frequency and residual height on quality parameters of two pasture types.

Treatment			OMD (% DM)	N (% DM)	CP (% DM)	ADF (% DM)	NDF (% DM)	Lignin (% DM)	OM (% DM)	ME (MJ ME/kg DM)
Mixture	**Frequency (weeks)**	**Height (mm)**								
Dairy	**3**	**25**	78.4 ± 4.97[1]	3.5 ± 0.11	22 ± 0.72	22.4 ± 2.59	33.4 ± 3.09	8.7 ± 1.47	89.3 ± 1.09	11.2 ± 0.74
		50	79.2 ± 7.03	3.5 ± 0.16	21.7 ± 1.02	21.7 ± 3.66	31.5 ± 4.37	8.8 ± 2.07	89.2 ± 1.55	11.3 ± 1.04
		75	78.5 ± 7.03	3.4 ± 0.16	21.1 ± 1.02	22.9 ± 3.66	33.6 ± 3.66	9.4 ± 2.07	89.2 ± 1.55	11.2 ± 1.04
		100	78.3 ± 7.03	3.3 ± 0.16	20.8 ± 1.02	22.2 ± 3.66	32.2 ± 3.66	8.6 ± 2.07	89.3 ± 1.55	11.2 ± 1.04
	9	**25**	75.4 ± 7.03	3.6 ± 0.16	22.5 ± 1.02	24.9 ± 3.66	35.5 ± 3.66	10 ± 2.07	89 ± 1.55	10.7 ± 1.04
		50	73.5 ± 7.03	3.8 ± 0.16	23.4 ± 1.02	26.3 ± 3.66	38.1 ± 3.66	12.3 ± 2.07	86.5 ± 1.55	10.2 ± 1.04
		75	74.2 ± 7.03	3.7 ± 0.16	22.9 ± 1.02	26.9 ± 3.66	39 ± 3.66	11.8 ± 2.07	86.2 ± 1.55	10.2 ± 1.04
		100	74.6 ± 7.03	3.8 ± 0.16	23.6 ± 1.02	24.2 ± 3.66	33.6 ± 3.66	9.8 ± 2.07	88.9 ± 1.55	10.6 ± 1.04
Sheep	**3**	**25**	72.7 ± 7.03	3.5 ± 0.16	21.8 ± 1.02	23.7 ± 3.66	35.6 ± 3.66	10 ± 2.07	89.2 ± 1.55	10.4 ± 1.04
		50	72.3 ± 7.03	3.5 ± 0.16	21.7 ± 1.02	24.7 ± 3.66	35.6 ± 3.66	10.4 ± 2.07	89 ± 1.55	10.3 ± 1.04
		75	72.5 ± 7.03	3.5 ± 0.16	22.2 ± 1.02	25.7 ± 3.66	37 ± 3.66	11.5 ± 2.07	89.5 ± 1.55	10.4 ± 1.04
		100	72.6 ± 7.03	3.7 ± 0.16	22.8 ± 1.02	25.8 ± 3.66	36.5 ± 3.66	11.1 ± 2.07	89.6 ± 1.55	10.4 ± 1.04
	9	**25**	70.9 ± 7.03	3.5 ± 0.16	21.9 ± 1.02	24.4 ± 3.66	36.1 ± 3.66	10.4 ± 2.07	89.6 ± 1.55	10.2 ± 1.04
		50	73.5 ± 7.03	4 ± 0.16	24.8 ± 1.02	26 ± 3.66	37.6 ± 3.66	11.7 ± 2.07	87.4 ± 1.55	10.3 ± 1.04
		75	72.2 ± 7.03	3.8 ± 0.16	23.4 ± 1.02	27.6 ± 3.66	38.9 ± 3.66	13.2 ± 2.07	84.3 ± 1.55	9.8 ± 1.04
		100	71.5 ± 7.03	3.4 ± 0.16	21.5 ± 1.02	25.7 ± 3.66	37.2 ± 3.66	11.1 ± 2.07	89.2 ± 1.55	10.2 ± 1.04

[1]result ± standard error

CHAPTER 5. DISCUSSION

Botanical composition analysis indicated that white and red clover, perennial ryegrass and plantain were the dominant species in the current study. More importantly, it was found that these dominant species were not sensitive to the range of defoliation treatments imposed over the 7-month study period. Detailed botanical composition by species was assessed at 3 time points throughout the seven-month study period. These 4 dominant species are commonly regarded as having higher phenotypic plasticity compared to others in the mixtures, whereby they produce the most favourable phenotypes for maximising resource acquisition and tolerating unfavourable conditions through different mechanisms (Sultan, 2004; Richards et al., 2006; Richardson and Pyšek, 2006; Davidson et al., 2011), thus making them more likely to establish successfully and to become a dominant species (Baker, 1965; Pigliucci, 2002; Liao et al., 2015). Phenotypic plasticity is an important trait, especially with changes predicted in future climate conditions (Valladares et al., 2014). Invasive species tend to exhibit greater plasticity than native and non-invasive species (Leicht-Young et al., 2007; Funk, 2008). Plantain, for example, is a species that agronomists formerly thought of as a weed, which has been commercially bred for pastoral use with a vertical growth habit compared to the natural ecotypes. Plantain has remained one of the dominant species throughout the current study, and has been shown to withstand varying residual height and interval treatments in other studies (Li et al., 1994; Ayala et al., 2011), especially at lower residual heights (Labreveux et al., 2004; Powell et al., 2007) and shorter defoliation frequencies (Cranston et al., 2021). In particular, plantain reverts to a prostrate growth profile under lower residual heights (Stewart, 1996). Similarly, white clover (particularly the smaller-leaved cultivars) has been shown to develop small-leaved, prostrate plants at lower residual heights (King, 1963), with leaves increasing in size as residual height increases (Brock and Hay, 2001), as long as one leaf remains below grazing height. However, larger-leaved cultivars may not be able to survive more frequent, closer grazing because of their larger and more upright leaves, as was apparent in the current study. Perennial ryegrass is considered to have a similar degree of plasticity as white clover (Brock and Hay, 1996), which was also noted in the current study, with composition being maintained under lower residual heights and shorter defoliation frequencies. Perennial ryegrass also has a similar survival mechanism to white clover under lower residual heights, producing smaller tillers with more prostrate growth (Matthew et al., 1996). Traditionally, red clover has less plasticity than the other dominant species used in the current study (Black et al., 2009), although recent plant breeding and development have improved this trait, resulting in semi-prostrate cultivars, such as the one used in the current study. However, the mechanism by which red clover adapts to close defoliation is smaller-sized petioles, with leaf size remaining the same (Gross et al., 2021). Birdsfoot trefoil has low plasticity, especially under low residual heights (Stewart, 2022), which was also evident in the current study, with birdsfoot trefoil being mostly absent under shorter defoliation frequencies and lower residual height treatments. It has also been established that chicory prefers higher grazing residuals (Clark et al., 1990; Li et al., 1997b), but no trends were observed in the current study. Crimson clover (*Trifolium incarnatum* L.) also prefers higher grazing residuals (Stewart, 2022), which was also found in the current study, although this effect had disappeared toward the end of the study.

The timing of sowing (5th May 2022, autumn in New Zealand) may have had an impact on the number of species present, and their contribution to overall yield. The ideal time for sowing most temperate grass and legume species is early autumn or late spring due to the soil temperatures required for germination (Hampton et al., 1999). In particular, spring sowing is recommended for chicory and plantain (Stewart, 2022). If chicory is planted in autumn, it is subject to vernalisation in the winter months, which induces reproductive growth the following summer. This occurred in the current study, with flowering stems present over several defoliation events, which would have negatively impacted herbage quality (Hodgson and Brookes, 1999). The recommended sowing time for cocksfoot, tall fescue, and meadow fescue is either late spring to early autumn, or early to mid spring, whilst the recommended time for Persian, balansa, arrowleaf and crimson clovers is autumn (Stewart, 2022). Also, white clover requires soil temperatures greater than 14°C (Moot et al., 2000), and so is suited to early autumn or spring sowing. Therefore, autumn sowing, which was used in the current study as it is the most common time to sow pastures in New Zealand, might be expected to result in staggered germination of many of the species in the hyper-diverse seed mix used. For example, timothy (*Phleum pratense* L.) requires a high thermal time for successful establishment (Moot et al., 2000), and so with the additional stress of shorter defoliation frequencies (3 and 6 weeks), did not survive in the current study.

No individual combination of treatments led to a significant increase in the number of species present at the end of this study, however at either end of the extreme treatments (shorter interval with lower residual height and longer interval with higher residual height), there was typically a lower number of different species present (see Table 15). There were also a number of species that were mostly absent throughout the entire study, including arrowleaf clover (*Trifolium vesiculosum* Savi.), balansa clover (*Trifolium michelianum* Savi.), crimson clover, lucerne, Persian clover (*Trifolium resupinatum* L.), phalaris, sanfoin, strawberry clover, subterranean clover (*Trifolium subterraneum* L.), tall fescue, timothy and hairy vetch. Species such as white clover, plantain, red clover and perennial ryegrass were present in all plots throughout the entire study, and cocksfoot was present in almost all plots throughout the study. Table 15 also shows that the hyper-diverse mixture reverted to a smaller number of dominant species within a short timeframe, questioning whether a simpler pasture mixture would have been easier and cheaper from the outset.

Table 15: Average number of species present in individual treatments [frequency (weeks) – residual height (mm) over all diverse pasture mixtures (dairy and sheep mixtures combined).

Treatment [frequency (weeks) – height (mm)]	Species present
3-25	8
3-50	8
3-75	8
3-100	8
6-25	9
6-50	9
6-75	9
6-100	9
9-25	9
9-50	9
9-75	8
9-100	8

Increasing the cutting residual height (from 25mm to 100mm) increased the legume fraction by approximately 15% (Appendix 5, Table 21) over both pasture mixtures. This functional group was mainly comprised of red and white clovers. While there were no significant differences between dominant species, there was a trend for increasing cutting residual height (i.e. higher residual) to reduce white clover percentage and increase red clover percentage. This is consistent with reports of red clover having poor performance under close grazing (Brock and Kane, 2003; Stewart, 2022). Longer cutting frequencies (i.e. less frequent) resulted in an increase in both the legume and dead matter components of pasture mixtures. However, there is a trade-off between longer rotation improving plant presence/persistence and at the same time increasing dead matter (along with stem), which would be expected to negatively impact quality (Lee et al., 2007; Stakelum and Dillon, 2007). With lower cutting residual heights and shorter cutting frequencies, it was common for perennial ryegrass and white clover to dominate pasture composition. This suggests that management of more diverse pastures needs to be altered to target the growth of selected individual species, or groups of species, as they are likely to have different optimal management.

Overall, the current study showed that under more frequent defoliation (i.e. three and six weeks), a higher residual height mostly resulted in an increased yield (except for 6 weekly-100 mm), as shown in Figure 8 (which is a graphical visualisation of the data from Table 13). This graph also shows that under more lax grazing (nine-week defoliation interval), a higher residual height generally resulted in a reduction in yield (except for 9 weekly-100 mm).

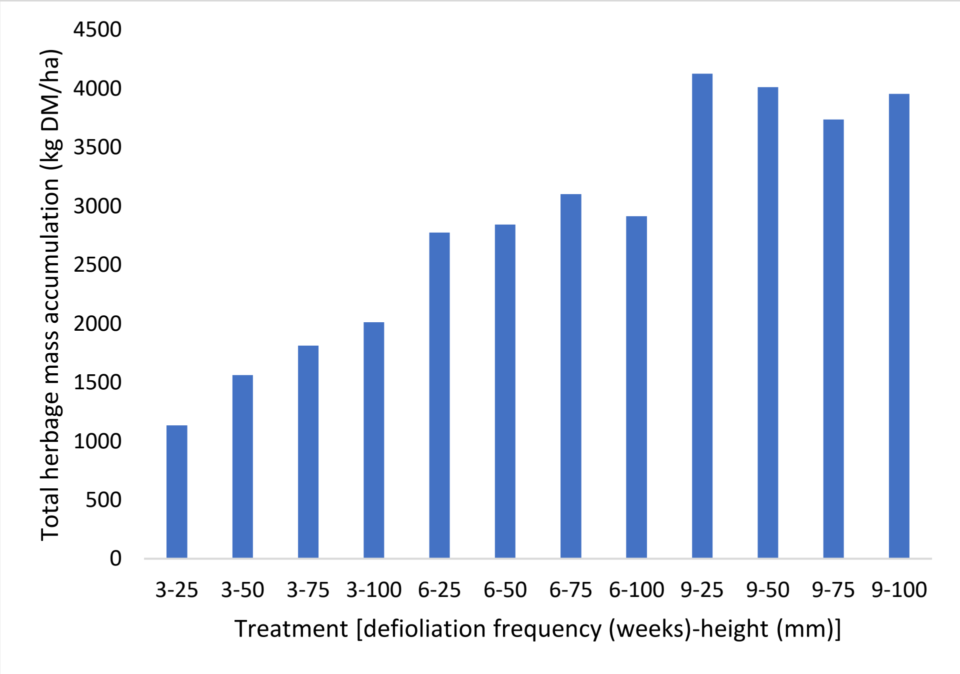

Figure 8: Total herbage accumulation (kg DM/ha) of hyper-diverse pastures under defoliation frequency (3, 6, and 9 weeks) and residual height (25, 50, 75, and 100 mm) treatments.

The sheep pasture mixture in this study showed that regardless of defoliation interval, a higher defoliation residual height resulted in increased yield (except for 6 weekly-75 mm and 9 weekly -75 mm), as seen in Figure 9 (which is another graphical visualisation of the data from Table 13). The same general trend was also found in a study by Cranston et al. (2021) with slightly different residual heights. Whilst the three-week defoliation interval in the dairy mixture showed an increased yield with higher defoliation residual heights (see Figure 9), the opposite was seen for the six- and nine-week defoliation frequencies (except for 6 weekly-75 mm). All interactions between defoliation residual height and interval were significant in the present study.

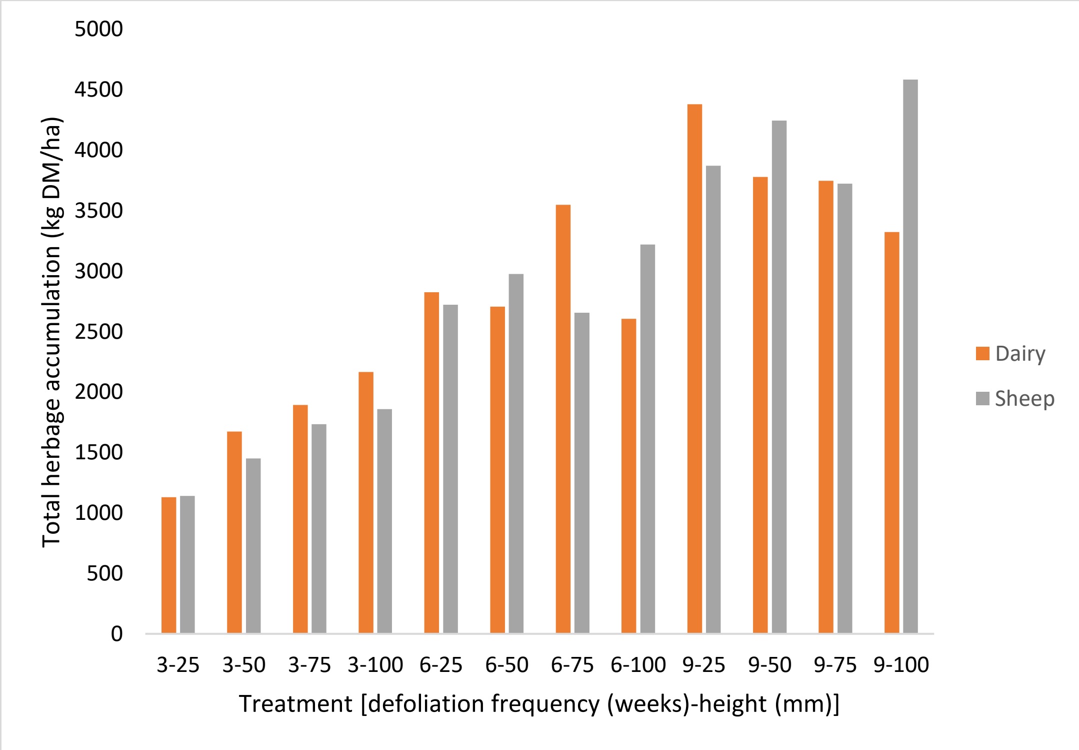

Figure 9: Total herbage accumulation (kg DM/ha) of hyper-diverse pastures (sheep and dairy pasture mixtures) under defoliation frequency (3, 6, and 9 weeks) and residual height (25, 50, 75, and 100 mm) treatments.

Along with environmental conditions and soil fertility, defoliation interval is one of the main factors affecting the regrowth, herbage yield, and quality of pasture. In particular, defoliation interval influences the storage and mobilisation of water-soluble carbohydrates (WSC) in grasses (Savitch et al., 1997; Xue et al., 2008; Cajarville et al., 2015), where too short a defoliation interval will not allow replenishment of enough WSC, which leads to reduced persistence and slower regrowth (Fulkerson and Donaghy, 2001; Li and Kemp, 2005; Lee et al., 2008). The present study found that longer defoliation frequencies generally increased yield, via a longer recovery period for plants between defoliation events. This effect was reported from multiple studies in a review by Roche et al. (2017), where principles of pasture management across 100 years of literature were collated. This relationship has held across multiple species (generally tested as single species, and occasionally as mixed pastures but only containing several species), and many years of research under both cutting and grazing. Weinmann (1948) proposed some time ago now, that an adequate rest period between defoliations was required for different grass species to replenish their WSC stores that had been used in regrowth. This has also been shown in a more-recent study by Cranston et al. (2015b), where they found an optimum recovery period to be 3 to 4 weeks, with no grazing over the winter months, for a herb and legume mixture (red and white clovers, plantain, and chicory). This is also supported by (Baker et al., 2023c), who found a 4-week defoliation interval resulted in increased yield for a grass, legume, and herb mixture (perennial ryegrass, timothy, white and red clover, plantain and chicory) compared to a 3-week interval. It has also been suggested that longer defoliation intervals (between 3 and 6 weeks) are required to maintain high red clover, and chicory levels within diverse mixtures (Cranston et al., 2021). However, species such as chicory may require shorter defoliation intervals during late spring and summer to control reproductive stems and maintain quality, as mentioned previously. Li et al. (1997a) found that chicory could maintain high growth rates under a defoliation interval of 2-3 weeks, and that longer intervals during the late spring and summer period provided poor control of reproductive stems and subsequent quality. Therefore, whilst many studies have provided details on adequate recovery periods for diverse pastures, this is dependent on species composition and which species the management is targeting, and changes on a seasonal basis.

Defoliation residual height has the potential to impact herbage quality, as the increased quantity of pasture (as observed under higher defoliation residual heights) contains more stem and dead material (Lee et al., 2007; Stakelum and Dillon, 2007). Yield responses from residual height may differ between species and would be more important over periods of stress (e.g. dry summers for some perennial grass species) (Brougham, 1956; Harris, 1971; Kemp et al., 2000). Lower residual heights can lead to smaller plants and a shorter root length, reducing their capacity to acquire nutrients, thus reducing their stress tolerance (Sullivan et al., 2000), especially under conditions of low soil moisture and low soil nutrient levels. However, in mixtures, the impact of defoliation residual height may affect certain species differently, especially when grazing is involved (e.g. selective grazing of different species). Defoliation residual height is expected to have a significant impact on the regrowth and survival of grasses by varying the amount of WSC resources available per tiller (Fulkerson and Sinclair, 2003).

It has been suggested that lower defoliation residual heights (<30 mm) favour species with similar growth habits to white clover and broadleaf species, whereas higher residual heights are optimal for species with more upright growth habits, such as most pasture grasses and herbs like narrow-leaved plantain and chicory (Lee et al., 2007; Ayala et al., 2011; Lee et al., 2015). Long-term defoliation at lower residual heights can lead to reduced yield (Leafe and Parsons, 1983; Hernández Garay et al., 2000), tiller density (Hernández Garay et al., 2000; Lee et al., 2007) and root growth (Evans, 1971; Hernández Garay et al., 2000; Lee et al., 2007). This may result in an increased reliance on WSC reserves (Davidson and Milthorpe, 1965; Fulkerson and Donaghy, 2001), a requirement for a longer subsequent defoliation interval for full recovery, and an increased risk of failure under adverse climatic conditions (Chapman, 2016; Donaghy et al., 2021). It has also been suggested that higher defoliation residual heights (>80 mm) result in reduced photosynthesis from the reduced leaf area index, which could be attributed to the development of pseudostem. The reduced photosynthesis could also result from an increase in the percentage of older leaves in the pasture under higher residual heights (Hernández Garay et al., 2000). More lax grazing with higher residual heights has also been shown to increase the proportion of stem in many species including chicory (>80%), and result in reduced growth rates (Matthews et al., 1990). Higher defoliation residual heights have also been shown to result in elevated stolons in white clover plants in an attempt to follow light within the pasture canopy during late spring to early summer, which also reduces root growth (Brock, 2006).

Cranston et al. (2015b) suggested that the optimal residual height for legume and herb mixtures is approximately 8 cm. There are a number of studies also noting an increased yield and persistence of both legume and herb species, particularly red clover and chicory, under higher grazing residual heights (Clark et al., 1990; Li et al., 1997b). The present study supports these previous findings, with an increase in both legume and herb functional groups under the higher defoliation residual heights (75mm and 100mm). Notably, there was a significant increase in red clover composition, in agreement with previous studies that this species does not perform well under lower residual heights (Brock and Kane, 2003; Stewart, 2022). It is important to note that due to this being a mowing study, it is not necessarily representative of animal grazing and associated behaviours (e.g. urination/defecation, treading, preferential grazing). However, the basic morphological and physiological processes in the plant are expected to be similar to what they are under grazing.

It is worth noting that these previous recommendations for higher residual heights are for mixtures excluding grass species, and the incorporation of grasses in mixtures including those used in the present study may have a negative impact on total yield, quality, and composition when defoliated to these higher residual heights. A number of studies have shown that perennial ryegrass yield is increased with lower defoliation residual height and longer defoliation frequencies (Reid, 1966, Clark et al., 1974, Fulkerson and Michell, 1987), which was also shown in the present study. However, Kerrisk and Thomson (1990) found that a higher defoliation residual height during summer, spring and autumn increased the yield of perennial ryegrass, phalaris and tall fescue. Their study showed that during winter, irrespective of residual height, there was no difference in the yield of perennial ryegrass and phalaris, although a higher residual height increased the yield of tall fescue (Kerrisk and Thomson, 1990). However, whilst a higher residual height may have a positive impact on grass yield, it is also associated with an increase in the proportion of plant stem and dead material, and thus reduced digestibility (Michell et al., 1987; Stakelum and Dillon, 1990; Pembleton et al., 2017) leading to reduced pasture utilisation (Wales et al., 1999; Moate et al., 2000), compared with a more optimal residual height (e.g. 40-50mm for most temperate grasses (Fulkerson and Donaghy, 2001)).

There were not enough data points to make any conclusions regarding the impacts of the defoliation treatments on seasonal yield. However, from the more frequent rising plate meter data, there was a higher yield during summer compared to autumn, except for a few treatments. Table 3 (page 21) shows that many studies have found diverse pasture mixtures to have increased yield over summer. This finding is not surprising, given that a number of species in this study are summer active, for example chicory and red clover (Brown et al., 2000), and plantain (Stewart, 1996; Labreveux et al., 2006; Moorhead and Piggot, 2009). In addition, Table 1 (page 18) also shows that some species in these mixtures have a higher proportion of their annual yield during summer (e.g. phalaris, cocksfoot, and white clover) (Kemp et al., 1999). With climate change expected to result in an increase in the frequency of extreme weather events, species that are capable of growing under these environments, especially warmer and drier conditions, will be a key factor to maintain productivity of temperate pastoral systems. This may be important to maintain, or even improve, lamb growth over the summer period prior to their slaughter (Powell et al., 2007), and to reduce the amount of supplementary feed required in dairy systems (Goh and Bruce, 2005).

However, it should be noted that the large yields recorded by the rising plate meter in the present study, along with bolting plants, mean that this tool may not be suitable to measure yield in hyper-diverse pastures using standard equations. Although, the use of a longer rising plate meter rod and calibration during different morphological stages of plant development may improve this tool for yield estimation in these pastures. Alternatively, other methods could also be developed to measure yield in hyper-diverse pastures. Quadrat cuts would be more appropriate for such research, but commercial farms might benefit more from techniques like pasture height estimated through hyperspectral imaging or remote sensing technologies, assuming calibration can be achieved for these methods.

There was no significant difference in quality parameters under any treatments in the present study, likely due to the small amount of data. However, there was a trend where higher defoliation residual heights resulted in increased lignin and NDF content, and lower defoliation residual height resulted in an increase in ME. This supports previous studies stating that defoliating pastures at higher residual heights are associated with an increased proportion of stem and dead material, potentially having a negative impact on pasture quality (Michell et al., 1987; Stakelum and Dillon, 1990; Pembleton et al., 2017). In saying that, regardless of defoliation residual height and grazing interval, the quality (specifically CP, NDF, and ME) of both mixtures when measured in May of 2023 was consistent with other literature and is sufficient to maintain high animal performance (Table 7) (Litherland and Lambert, 2017).

CHAPTER 6. CONCLUSION

The results of this study indicate that, while hyper-diverse pastures were comprised of a large number of species at sowing (18 and 19 species in the dairy and sheep diverse mixtures, respectively in this study), not all remained productive together, or throughout time. In this case, approximately 8-9 species were present within the pasture over the relatively short 7-month experimental period. Of those species that remained productive, only several were dominant at any given time. Again, in the present study, there were 4 dominant species (perennial ryegrass, white clover, plantain, and red clover) that appeared to be not sensitive to any specific defoliation residual height or interval treatment.

As this is one of the first studies investigating hyper-diverse mixtures, there was scant literature looking at the establishment of mixtures comprising a multitude of species over a prolonged period. Further research is required to identify timing and methods for successful establishment of hyper-diverse pasture mixtures (i.e. separate or sequential summer vs. autumn sowing). Once this has been identified, the next logical step is to carry out further detailed studies on the effect of defoliation management, particularly regarding the impact on pasture quality and persistence over a longer period of time.

The reversion of the hyper-diverse mixture to a smaller number of dominant species within such a short timeframe, poses the question of whether it would have been easier to create a simpler pasture mixture at the outset, with significant savings in seed and sowing costs. However, the survival and dominance of species was undoubtedly driven by edaphic conditions, along with the prevailing climate at time of sowing and throughout the experimental period, and interactions between these and the defoliation management. Therefore, more research needs to be undertaken before clear recommendations are able to be made regarding likely suitable situation-specific mixtures, and these will vary with region and management system (e.g. dairy, sheep, beef). However, regardless of management, this study reports that this pasture mixture had adequate quality and yield to maintain high animal performance, indicating that it has the potential to be successful in New Zealand pastoral systems, in the short term at least.

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APPENDICES

APPENDIX 1.

Table 16: Characteristics of grass, legume, and herb species reported in the literature (adapted from Vibart et al. (2016)).

	Characteristics							
Species	Rooting depth (m)	NDF (g/ kg DM)	Total N (g/ kg DM)	NO3 (g/ kg DM)	CP (g/ kg DM)	ME (MJ/ kg DM)	DM digestibility (g/ g DM)	CT (g/ kg DM)
Perennial ryegrass	0.75 - 1.5	489-552	2.6	1.1-3.5	221-263	9.9-11.7	0.71-0.74	0-1.8
Tall fescue	0.89-1.5	488-554	2.5	1.4-3.8	216-269	9.9-10.6	0.7-0.76	-
Cocksfoot	0.77-1.47	485-594	2.7	1.1-3.2	228-319	9.7-11.2	0.7-0.74	-
Phalaris	0.56-1.94	417-553	-	1.5-3.2	241-291	10.2-11	0.7-0.74	-
Prairie grass	0.82-1.5	437-555	-	0.9-2.8	246-310	10.7-11.2	-	-
Kikuyu	1.27-2.01	503-639	-	1.7-5.2	228-293	9.2-9.5	-	-
Paspalum	0.47-1.5	604-672	-	1.4-1.7	198-229	8.9-9	-	-
White clover	1.47-1.5	276-339	3.36-4.55	0.7-0.8	206-298	9.3-10	-	3.1
Red clover	1.13-1.5	342-476	2.81-4.3	0.4-0.8	166-303	8.3-10	0.76	1.7
Alfalfa	1.46-1.95	314-472	3.06	0.2-1.3	206-301	9.0-11	0.71	0
Chicory	1.40-1.71	127	1.72	-	123-244	13.7	0.79	4.2
Plantain	0.64-1.5	312-327[1]	1.6	-	183-203[1 2]	11.1[2]	0.84-0.88[1]	14

[1]Labreveux et al. (2006), [2]Rodriguez-Firpo et al. (2022).

APPENDIX 2.

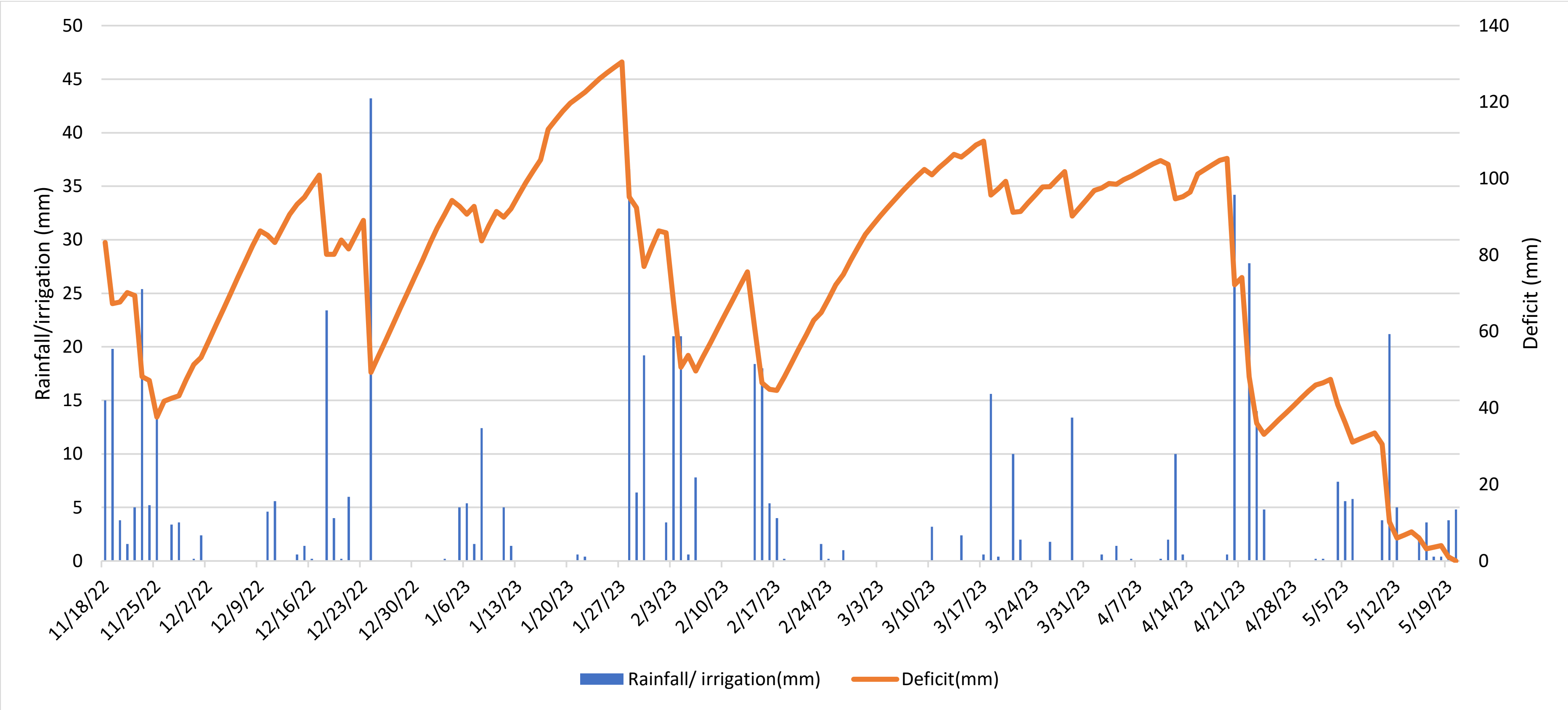


Figure 10: Soil water balance graph.

APPENDIX 3.

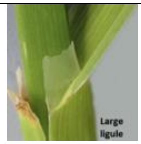
Table 17: Sampling frequency dates (from 8th November 2022 to 8th June 2023).

		SAMPLING		
WEEK	**Date**	**3-weekly**	**6-weekly**	**9-weekly**
0	**8-Nov**	63.5 mm cut	63.5 mm cut	63.5 mm cut
1	**15-Nov**			
2	**22-Nov**			
3	**29-Nov**	Yield/Botanical		
4	**6-Dec**			
5	**13-Dec**	Visual Botanical Samples		
6	**20-Dec**	Yield/Botanical	Yield/Botanical	
7	**27-Dec**			
8	**3-Jan**			
9	**10-Jan**	Yield/Botanical		Yield/Botanical
10	**17-Jan**			
11	**24-Jan**			
12	**31-Jan**	Yield/Botanical	Yield/Botanical	
13	**7-Feb**			
14	**14-Feb**			
15	**21-Feb**	Yield/Botanical		
16	**28-Feb**	Visual Botanical Samples		
17	**7-Mar**			
18	**14-Mar**	Yield/Botanical	Yield/Botanical	Yield/Botanical
19	**21-Mar**			
20	**28-Mar**			
21	**4-Apr**	Yield/Botanical		
22	**11-Apr**			
23	**18-Apr**			
24	**25-Apr**	Yield/Botanical	Yield/Botanical	
25	**2-May**			
26	**9-May**	Visual Botanical Samples		
27	**16-May**	Yield/Botanical/ Residual Cut		Yield/Botanical/ Residual Cut
28	**23-May**			
29	**30-May**			
30	**6-Jun**		Residual Cut	

APPENDIX 4.

Table 18: Pasture identification resource: grasses [adapted from (Southward and Harrington, 2023)].

Grass Species		Hair	Ribbed	Ligule	Auricles	Shiny/Matte	Colour	Seed Head	Silica Teeth	Tiller base colour	Tillers folded / rolled	Other
												
Browntop (*Agrostis capillaris*)		Hairless	Ribbed Midrib almost invisible 	Very short Inconspicuous in side view		Matte	Bluish green colour	Small open branched panicles One floret per spikelet 	N/A	N/A	Rolled	Short and wide
Cocksfoot (*Dactylis glomerata* L.)		Hairless and smooth	No ribbing	Length 2-12mm Membranous Sydney opera house roof-shaped	N/A	Matte 	Dull, blueish green	Large and bushy 	Silica teeth on leaf margin 	N/A	Very strongly flattened	Leaves pointed with 'boat bow' tip

												
Meadow fescue (*Festuca pratensis* Huds.)		Hairless	Ribbed upper surface	Length 0.5mm Membranous Greenish Truncate to obtuse	Length 1.5 mm Claw-like or blunt Yellow-green to creamy - white 	Upper surface dull Lower surface glossy	Bright green		N/A	Reddish to purple	Emerging leaf rolled	Rhizomes Sheath pale green Split near base Sheath margins overlapping
Perennial ryegrass (*Lolium perenne* L.)		Hairless	Ribbed (upper surface to leaf blade) 	Length 1-2mm Membranous Mainly hidden 	Short Inconspicuous Claw-like 	Shiny lower surface 	Deep emerald green	Flattened spikelets alternating up stem. No awns. 	N/A	Red, sometimes only slightly 	Emerging leaf folded	Narrow leaves Leaf blades pointed

Phalaris (*Phalaris aquatica* L.)		Hairless	Upper surface ribbed	Length 5-10mm Membranous Pointed Often overlapping around stem 	N/A	Matte	Bluish-green leaves	Cylindrical seed head 	N/A	Dark pinkish scaled leaves 	Rolled	Tough underground rhizomes Twisted leaves
Poa (*Poa annua*)		Hairless	No ribbing 	Membranous Serrated edge	N/A	Matte	Yellow-green	Open panicle 	N/A	N/A		Small plant Leaf blade often crinkled during elongation Canoe-shaped tip Prominent square groove at midrib Flowers appear at

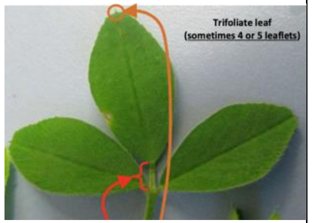
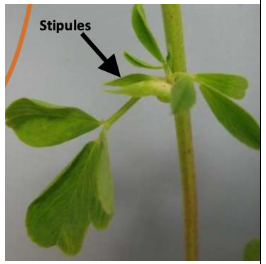
												any time of year
Prairie grass (*Bromus catharticus* Vahl.)		Hairy leaf sheaths, but hairless	Ribbed 	Length 3-5mm Membranous White Tapered toward toothed tip 	N/A	Matte	Pseudostem veins a little darker on light green background	Large open branched panicles with several seeds per spikelet Strongly folded 	Silica teeth on margins and lower leaf surface	N/A	Folded	Large leaved with large tillers
Tall fescue [*Festuca arundinacea* (Schreb.)]		Hairless and smooth	Ribbed upper surface (coarser than ryegrass) 	Length 0.5-3mm Firm Membranous	Clasping Small Hairy 	Glossy sheen on lower surface (less shiny than ryegrass)	N/A	Large branched panicles 	Fine silica teeth on leaf margin	Sometimes red	Emerging leaf rolled	Broad leaves stiff, flattened and narrowing to a point

													
Timothy (*Phleum pratense* L.)		Hairless	Indistinct midrib	Very short Dome shaped Membranous 	N/A	Matte	Dull blueish green colour	Dense Spike-like panicle Uniform shape along length 	Fine silica teeth on leaf margin	Creamy-white colour at the base of the pseudostem 	Rolled	Bulb-like swelling on internodes at stem bases Broad leaves Distinctive double-tipped spikelets

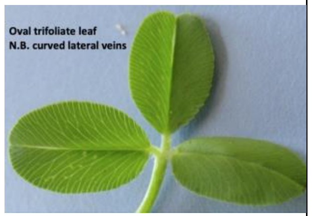
Table 19: Pasture identification resource: legumes [adapted from (Southward and Harrington, 2023)].

Legume Species		**Hair**	**Leaf shape**	**Flower/ seed head**	**Stipules**	**Other**
Arrowleaf Clover (*Trifolium vesiculosum*)		Smooth	Trifoliate Arrow-shaped Large 'V' watermark 	Large conical flower head White to pink flowers opening from bottom to top	White Long Narrow Pointed Prominent venation	Plant upright, glabrous. Stem hollow, and often purple
Balansa Clover (*Trifolium michelianum* Savi.)		Hairless	Trifoliate leaf varies in size and shape. Some have strong markings (can be white, silver, pink, purple or plain) 	Roundish flower, white to pinkish, 2-3 cm, each on separate stem Pods yellow-red 	Distinctive veined and pointed stipules 	Hollow-stemmed. Can grow over 0.5m tall, but remains prostrate when grazed. Semi-erect Multibranched Leaflet margins can be smooth or serrated Pointed ends of leaflets
Birdsfoot Trefoil (*Lotus corniculatus* L.)		Hairless	Trifoliate leaf on a short petiole with stipules shaped like leaflets Superficial appearance of a 5-foliate leaf	Yellow flower on stalks up to 15 cm long Mature pods arranged in birds foot pattern	Stipules shaped like leaflets	Long, trailing stoloniferous stems, up to 1 m long. More erect

				 		growing with no stolons.
Crimson Clover (*Trifolium incarnatum* L.)		Densely hairy	Trifoliate, Length 1.5-2.5 cm Egg to heart-shaped 	Red Inflorescence 	Elliptic Blunt Violet-veined Reddished tipped Mostly fused to stem 	Leaves finely toothed near tip
Hairy vetch (*Vicia villosa* Roth.)		Hairy	Even-pinnate Terminal tendril Pubescent, Elliptical to lance-shaped 	Purple to white inflorescence 	Small Pointed Feathery	Plant erect Long branching stems/vines Stem slender Vine-like Somewhat pubescent

Lucerne (*Medicago sativa* L.)		Hairless/hairy and without a leaf mark.	Trifoliate leaf Middle leaflet with longer stalk than lateral leaflets and midrib projects distinctly to a small point. At base of petiole (leaf stem), often another pair of small trifoliate leaves plus a pair of sharply pointed stipules Toothed along the edge (key difference to birdsfoot trefoil). 	Purple flower (occasionally yellow) Mature seed pod coiled to a spiral and black in colour  	Pair of sharply pointed stipules 	
Persian Clover (*Trifolium resupinatum* L.)		Hairless	Trifoliate, 1-3cm long Oval to oblong shaped Serrated leaflet edge	Pink inflorescence	Pointed Red venation	Stem hollow, Branching from base Plant prostrate/semi-erect Forms

						dense swards Rosette base
Red Clover (*Trifolium pratense* L.)		Hairy 	Roundish oval-shaped leaflets Trifoliate leaf. Typically large leaves Distinctive V-shaped leaflet mark 	Distinctive large red-purple inflorescence  	Relatively large stipules Distinctive reddish veins. Pointed up to one third of length 	Forms distinctive plants

Sanfoin (*Onobrychis viciifolia* Scop.)		Hairless	Pinnate leaf with elongated oval leaflets 	Pink flower with flat oval pods 	N/A	Plant around 40cm tall
Strawberry Clover (*Trifolium fragiferum* L.*)*		Hairless	Oval trifoliate leaf 	Pinkish in colour Swells to a round-shaped Seed head after florets die which resemble a dry strawberry.  	Stipules larger than white clover Encircle the stem & come to a long point. 	

Subterranean Clover (*Trifolium subterraneum* L.)		Hairy	Heart-shaped trifoliate leaves Often with oval dark purple or black spots/flecks between leaf lateral veins 	Small White flower with 2-5 florets on short stalks form seed burrs  	Stipules broad, veined and pointed for half their length 	Stems are prostrate, but do not root at node. Seeds are black and large
White Clover (*Trifolium repens* L.)		Hairless 	Trifoliate leaf Whitish v-shaped leaflet mark Each leaflet is slightly notched at the tip 	White flower Darkens to a brown seed head with 3-4 yellow to brownish irregularly heart-shaped seeds in each small pod	Small pointed stipules at base of petiole. 	Lateral leaflet veins straight Daughter plant forms stolons that grow along ground surface. Roots grow at nodes (may be buried in winter)

				Inflorescence Seedhead		Leaves & inflorescence growin from stolon (creeping stem)

Table 20: Pasture identification resource: herbs [adapted from (Southward and Harrington, 2023)].

Species		**Hair**	**Leaf shape**	**Ribbing/ veins**	**Flower/ seed head**	**Other characteristics**
Chicory (*Cichorium intybus* L.)		Hairless and smooth	Lance-shaped 	Lateral veins near perpendicular to main vein 	Purple flower 	Grows from basal rosette
Plantain (*Plantago lanceolata* L.)		Upward fine hairs	Lance-shaped flattened leaves 	Prominent parallel ribbing 	A ring of pale yellow anthers on a hairy stem. 	Grows from basal rosette

Sheep's Burnet (*Sanguisorba minor*)		Hairy	Concertina-shaped Each leaflet has toothed margins 	Defined mid-rib	Pink/red inflorescence 	Erect Tufted Basal rosette Stem red-brown Glabrous

APPENDIX 5.

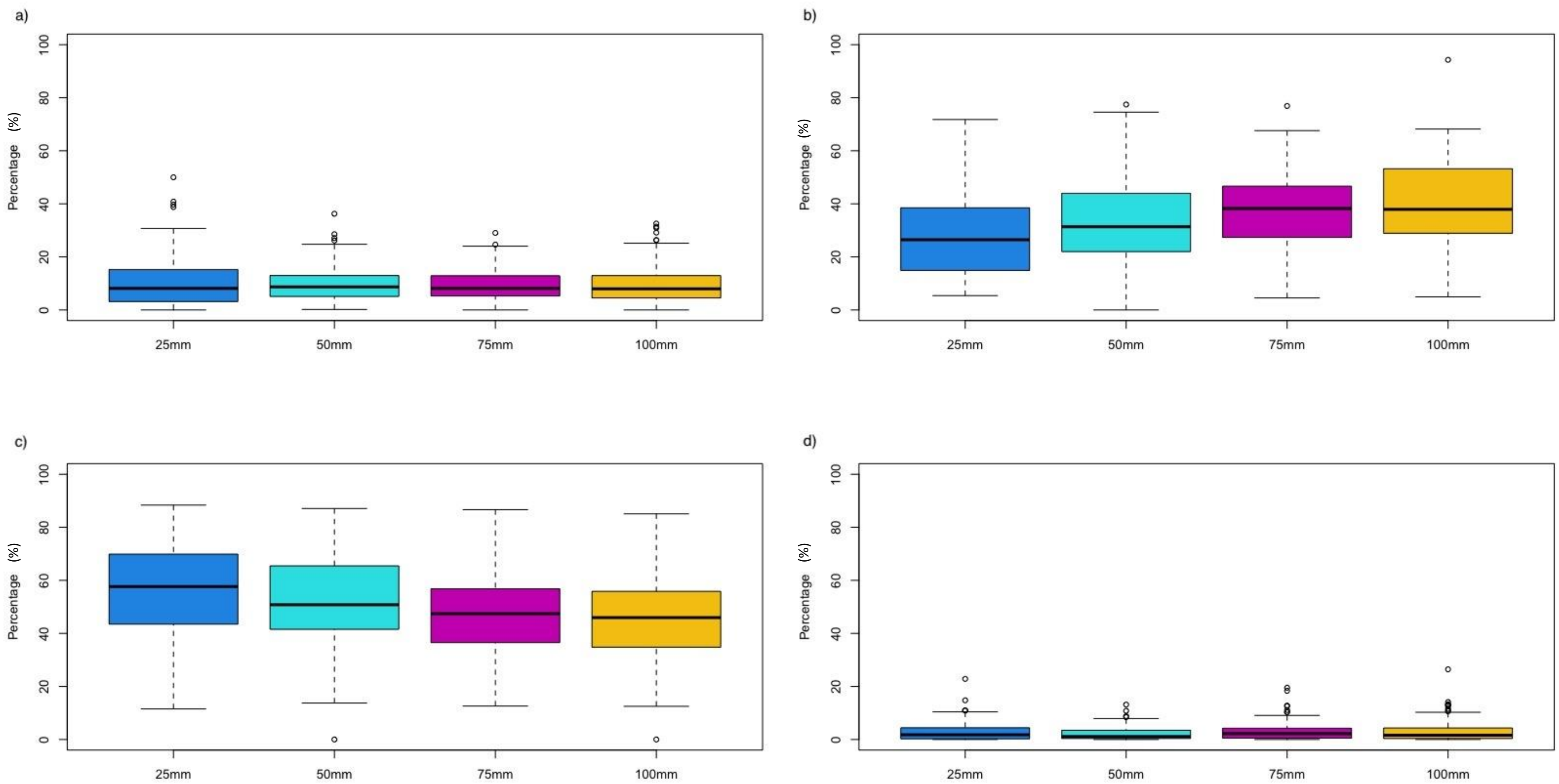


Figure 11: Botanical composition (functional groups) of diverse pastures following post-cutting residual heights of 25mm, 50mm, 75mm and 100mm shown by functional groups; a) grass, b) legume, c) herb, d) dead matter.

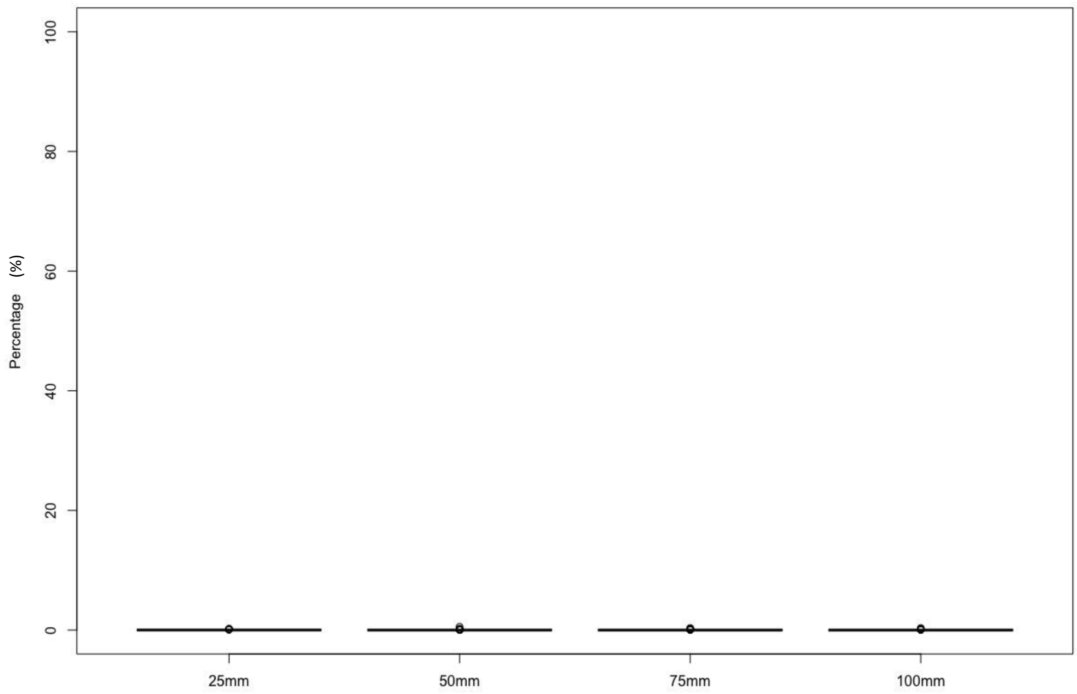


Figure 12: Botanical composition (functional groups) of diverse pastures following post-cutting residual heights of 25mm, 50mm, 75mm and 100mm shown by functional groups (non-sown fraction).

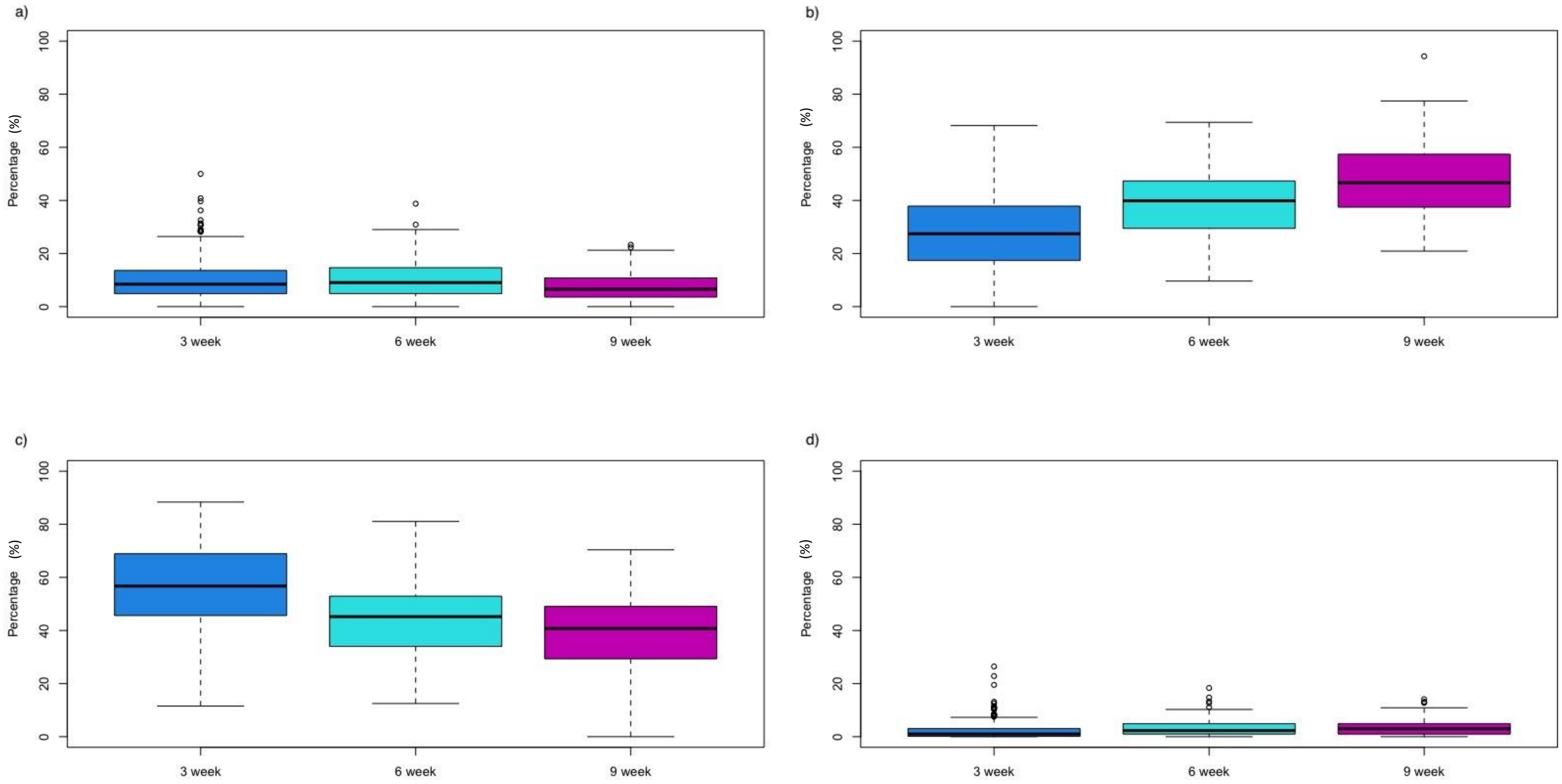


Figure 13: Botanical composition (functional groups) of diverse pastures following defoliation frequencies of three weeks, six weeks and nine weeks shown by functional groups; a) grass, b) legume, c) herb, d) dead matter.

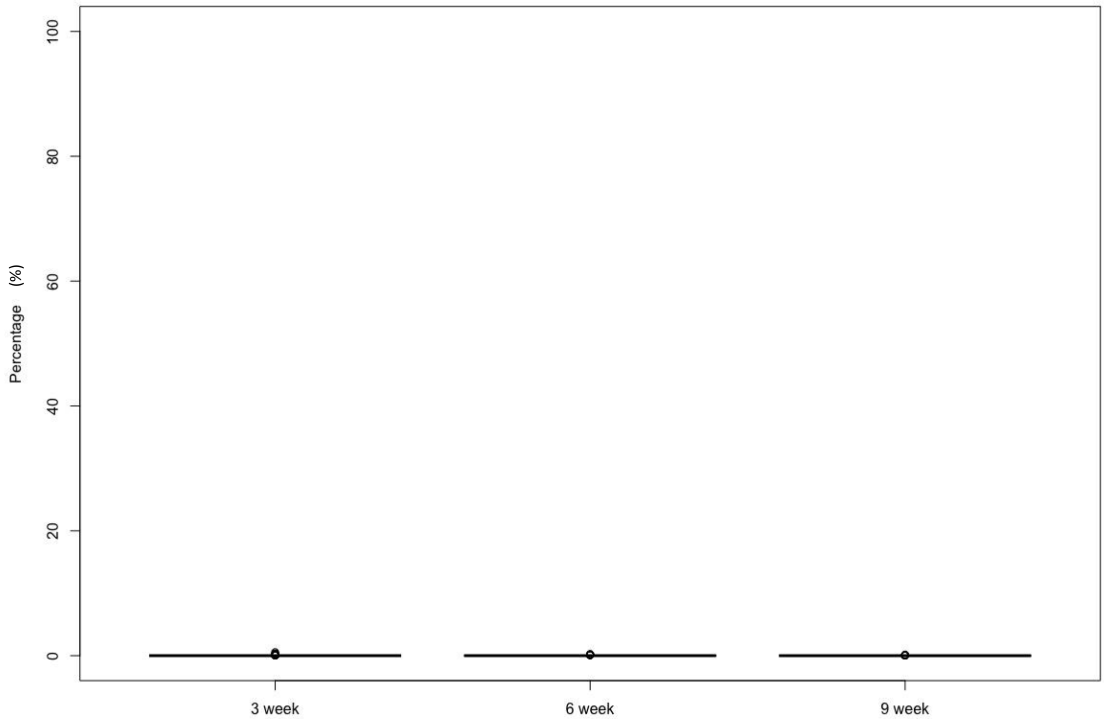


Figure 14: Botanical composition (functional groups) of diverse pastures following defoliation frequencies of three weeks, six weeks and nine weeks shown by functional groups (non-sown fraction).

APPENDIX 6.

Table 21: Effect of drying methods (62 vs. 95 vs. Freeze) on organic matter digestibility (OMD), N_p, N_pDM, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin (Lig), organic matter (OM), Ash, digestibility of organic matter in dry matter (DOMD), metabolisable energy (ME), soluble sugars (SS), starch, Cfat, non-structural carbohydrates (NSC). Least square means ± SEM.

Parameters	Drying Method			p value
	62	95	Freeze	
OMD (%DM)	71.1 ± 0.48^{b}	64.6 ± 0.48^{a}	88.5 ± 0.48^{c}	<0.001
N_p	3.57 ± 0.04^{b}	3.42 ± 0.04^{a}	3.28 ± 0.04^{a}	<0.001
N_pDM	3.69 ± 0.04^{b}	3.49 ± 0.04^{a}	3.38 ± 0.04^{a}	<0.001
CP (%DM)	23.0 ± 0.27^{b}	21.8 ± 0.27^{a}	21.0 ± 0.27^{a}	<0.001
ADF (%DM)	25.5 ± 0.38^{b}	29.1 ± 0.38^{c}	17.4 ± 0.38^{a}	<0.001
NDF (%DM)	37.2 ± 0.47^{b}	40.7 ± 0.47^{c}	26.6 ± 0.47^{a}	<0.001
Lig	9.29 ± 0.27^{b}	13.3 ± 0.27^{c}	7.38 ± 0.27^{a}	<0.001
OM	88.6 ± 0.14^{b}	89.5 ± 0.14^{a}	89.6 ± 0.14^{a}	<0.001
Ash	11.4 ± 0.14^{b}	10.5 ± 0.14^{a}	10.4 ± 0.14^{a}	<0.001
DOMD	63.0 ± 0.46^{b}	57.9 ± 0.46^{a}	79.3 ± 0.46^{c}	<0.001
ME (MJ/kg DM)	10.1 ± 0.07^{b}	9.26 ± 0.07^{a}	12.7 ± 0.07^{c}	<0.001
SS (%DM)	9.50 ± 0.30^{a}	10.5 ± 0.30^{b}	16.3 ± 0.30^{c}	<0.001
Starch (%DM)	2.19 ± 0.12^{a}	2.68 ± 0.12^{b}	2.45 ± 0.12^{ab}	0.021
Cfat	2.88 ± 0.07^{b}	2.64 ± 0.07^{a}	3.24 ± 0.07^{c}	<0.001
NSC	25.5 ± 0.50^{a}	24.4 ± 0.50^{a}	38.8 ± 0.50^{b}	<0.001

^{a,b,c} Means between columns with differing superscripts are different ($p < 0$)