LEAF REGROWTH STAGE AS A MORPHO-PHYSIOLOGICAL INDICATOR OF *BROMUS VALDIVIANUS* AND *LOLIUM PERENNE* MIXED PASTURE DEFOLIATION IN NEW ZEALAND GRAZING SYSTEM

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Plant Science

At Massey University, Manawatū, New Zealand

Javier Horacio García Favre

2022

ABSTRACT

Bromus valdivianus Phill. is a perennial grass species native to the South of Chile. Its better fitness for growing under low water availabilities compared with *Lolium perenne* L. has been assessed in field and glasshouse studies. However, *Bromus valdivianus* morpho-physiological attributes, such as root development under field conditions, and competitive ability are likely to manifest differently under contrasting defoliation criteria based on leaf regrowth stage. In addition, *Lolium perenne* and *B. valdivianus* growth can be complementary throughout the year under rainfed conditions. Therefore, the objective of the present thesis was to determine defoliation criteria based on leaf regrowth stage of mixed pastures (50/50% *L. perenne* and *B. valdivianus*) and consequences for species succession and functional traits development of the species. Four studies were developed in the thesis, two glasshouse and two field studies. The first glasshouse study evaluated the growth response and water-soluble carbohydrate (WSC) accumulation of *B. valdivianus* at three different defoliation frequencies. The results suggested that *B. valdivianus* increased herbage mass production and root biomass at low defoliation frequency (i.e., at 3.5–4.0 leaf regrowth stage). This was related to a higher WSC accumulation in the tiller base. In addition, under low soil water availabilities (20–25% of field capacity) WSC increased by ~20%, which indicated a drought resistant strategy of this species. The second study proved the growth enhancement (mainly root length and biomass) of *B. valdivianus* under competition with *L. perenne* compared with intraspecific competition when soil water shifted from high to low availabilities. In field studies, *B. valdivianus* mixed with *L. perenne* increased ~15% accumulated herbage mass compared to the species monocultures, which supported higher production during dry periods. This was due to the niche complementarity and asynchrony in herbage growth between the species, with *B. valdivianus* capable to maintain a steady tiller population throughout the year and root biomass accumulation at depth. Whereas *L. perenne* grew more than *B. valdivianus* under low level of oxygen in the soil and under optimal growth conditions and presented a higher nutritive value than *B. valdivianus* during winter, spring and autumn. The latter supports the partial grazing preference for *L. perenne* shown by sheep during part of the year, as measured in the second field study. This lower nutritive value (less energy) of *B. valdivianus* monocultures was overcame in the mixture, with a good overall value for high animal production. Regarding the mixture defoliation criterion, it was shown that it can be based on the optimal leaf regrowth stage of either species, as herbage mass production was similar between defoliation frequencies based on optimal leaf regrowth stage of either of the species. However, under defoliation based on optimal leaf regrowth stage of *B. valdivianus*, root biomass accumulation at depth increased ~45%. Overall, the results of the present thesis evidenced that *Bromus valdivianus* can successfully grow alongside *L. perenne* and, therefore, the mixture can increase forage production of New Zealand farming system in the event of climate change.

ACKNOWLEDGMENTS

I thank all the people that helped me through my studies. I specially express my gratitude to my main supervisor Ignacio López, who supported me and gave me full confidence that I was capable of fulfilling my commitments. His guidance and positivity made this journey gratifying. His critical and “outlier” point of view in pasture science helped me to grow as a scientific.

I express my sincere thanks to my co-supervisor Lydia Cranston for her excellent guidance and commitment into my PhD project. Lydia was always willing to help and solve any kind of problem. I appreciate her valuable edits and comments/suggestions to my manuscripts. To my co-supervisor Peter kemp, who wisely advised me from the day one. Peter’s suggestions greatly enhanced my studies and help me to comprehend the knowledge gap in pasture science. To my co-supervisor Danny Donaghy for his help during the whole period, for the suggestions and editing on my manuscripts and for tutoring when some ideas were a bit fuzzy, which significantly improved my studies.

I thank the technical staff from Massey, especially Mark Osborne, Brian Smith, Kay Sinclair and Chris Rawlingson for the support and goodwill to any work during the whole PhD period. I thank all the staff from Plant Growth Unit (PGU), especially Lesley Taylor for helping during my glasshouse studies. I also thank Iván Ordoñez for his welcome in the Massey environment and Yongmei Zhang for her help in my glasshouse studies.

I thank Martín and Manu, my New Zealand family, for opening their home to me. To Félix for all the nice talk about life. To Flor, Seba, Dany and Susi, who made Palmy less boring, and for letting me start a friendship with them.

Last but not least, I am also grateful to Cande for sharing this journey with me and for all the beautiful moments lived during these years. To my family: Marisa, Horacio, Elena, Stella, and Mónica, who always encourage me to go further. To Mabel, Brenda and Eric for all the weekend calls, weakening the remoteness. I thank Fede and Peru for their friendship and support no matter where I am in the world.

The financial assistance provided by Massey University Doctoral Scholarship, John Hodgson Pastoral Science Scholarship and Seed Tech Services Scholarship is acknowledged.

> “Para el que mira sin ver
>
> La tierra es tierra nomás
>
> Nada le dice la pampa
>
> Ni el arroyo, ni el sauzal”
>
> Atahualpa Yupanqui

Table of contents

List of figures

Glossary of Abbreviations

Abbreviation	**Full name/meaning**	**Unit**
Lp	*Lolium perenne* L.	
Bv	*Bromus valdivianus* Phil.	
Df	Defoliation frequency	
AGDD	Accumulated growing degree days	°C day
FC	Field capacity	%
LS	Leaf regrowth stage	
WSC	Water-soluble carbohydrate	mg $tiller^{-1}$
I	Irrigation	L m^{-2}
IC	Irrigation criteria	%
WC	Substrate water content	%
BD	Bulk density	mg m^{-3}
SD	Substrate depth	m
PA	Pot area	m^2
Wl	Soil water content	20–25% FC/80−85% FC
PCA	Principal component analysis	
PC1	Principal component one	
PC2	Principal component two	
SLA	Specific leaf area	mm^2 mg^{-1}

DM	Dry matter	%
CVA	Canonical variate analysis	
CAN1	Canonical variate one	
CAN2	Canonical variate two	
Mx	Mixture	
Mc	Monoculture of *B. valdivianus*	
MDA	Malondialdehyde	µmol L^{-1}
SEM	Standard error of the mean	
DMD	Dry matter digestibility	%
CP	Crude protein	%
NDF	Neutral detergent fibre	%
ADF	Acid detergent fibre	%
AM	Grazing after sunrise	
PM	Grazing before sunset	

CHAPTER 1

General introduction

1.1 INTRODUCTION

Global warming has increased the frequency of extreme climate events resulting in an increment in the variability of agricultural production. New Zealand is not exempt to these changes (Parry et al., 2007). Whereby since 1970 average air temperature has increased by 0.5 to 0.7°C, and summer soil water restriction has increased (Henessy et al., 2007). These changes are relevant for New Zealand pastoral farming systems, especially for rainfed systems in the eastern part of the country. Perennial ryegrass (*Lolium perenne* L.) is widely utilised in New Zealand grazing systems, mixed with white clover (*Trifolium repens* L.). It is a fast-growing species that provides high annual accumulated herbage mass (e.g., 15,000 kg DM ha-1 year^{-1} (Lee et al., 2012)). However, *L. perenne* has a shallow root system, thus during the summer, when soil water is restricted, *L. perenne* is under stress and displays a reduced growth rate (Hofer et al., 2016).

To mitigate the effect of reduced pasture growth during summer, farmers can use summer crops or other type of supplements, increasing the costs of production (Litherland et al., 2002). Hence, to enhance the performance of New Zealand pastures (during the soil water restriction period), the utilisation of pasture species with different morpho-physiological characteristics compared to traditional perennial ryegrass/white clover (*L. perenne/Trifolium repens*) sward are being considered.

Bromus valdivianus Phil. is a perennial grass species with growth requirements similar to those of *L. perenne* (López et al., 1997b), but with a better fitness for growing under low soil water availability (Keim et al., 2015). Therefore, it could complement the growth pattern of *L. perenne* throughout the year in a mixed pasture, supporting higher herbage mass production during summer and eventually a better persistence than the ryegrass/white clover mixes under future climates.

Chapman (2016) stated that the advantage of New Zealand pastoral farming systems is that they are based on continued improvements in total perennial grass harvested by animals, and more eco-physiological studies need to be done to understand this grazing process and enable continued improvement. The use of pasture mixes with greater diversity may allow for greater use of total soil resources (Ewel, 1991), however, more complex defoliation management may be required compared to ryegrass/white clover mixes. Defoliation criterion of pastures based on leaf regrowth stage supports high herbage production and nutritive value without penalties in the species persistence compared to other defoliation indicators, such as pasture height or pasture herbage mass (Fulkerson & Donaghy, 2001).

According to this general introduction and the following literature review (Chapter 2), the hypothesis tested in this thesis is that defoliation frequency based on pasture species leaf regrowth stage drives divergent species successional pathways and morpho-physiological states of the species in a mixed pasture.

Objectives of this thesis are as follows:

1) Determine optimal leaf regrowth stage of *Bromus valdivianus* based on water soluble carbohydrates accumulation in the tiller base. Quantify morpho-physiological variations of *Bromus valdivianus* exposed to different defoliation frequencies.

2) Determine species succession in a diverse pasture when it is defoliated at different leaf stage range, under New Zealand pastoral system conditions.

3) Evaluate the herbage mass accumulation in a *Bromus valdivianus* - *Lolium perenne* pasture, species domination through time, and the effect of soil water restriction on their respective growth. Determine the leaf regrowth stage equivalence between *Lolium*

perenne and *Bromus valdivianus* and the degree of concurrence and overlapping of their leaf stage range.

4) Determine *Bromus valdivianus* and *Lolium perenne* tiller growth dynamic and regrowth post-defoliation as monoculture and together. Evaluate the impact of this on components of the yield, herbage mass accumulation, herbage quality and species persistence.

5) Evaluate sheep preferential grazing between *Bromus valdivianus* and *Lolium perenne* during different seasons. Determine the morpho-physiological attributes that influence sheep preference between both grass species.

1.2 THESIS STRUCTURE

The present thesis is composed of seven chapters. The first chapter is the general introduction, Chapter 2 concepts and current knowledge on plant relationship, effect of pasture diversity on ecosystems and plant-animal interactions.

Chapters 3, 4, 5 and 6 are research chapters. Chapter 3 assessed *Bromus valdivianus* optimal leaf regrowth stage to enhance herbage mass and root biomass production. This is followed in Chapter 4 by the determination of *B. valdivianus* competitive ability to grow alongside *L. perenne* at different defoliation frequencies and soil water content availabilities. These studies were conducted in a glasshouse.

Chapter 5 and 6 were carried out under field conditions. Chapter 5 evaluated sheep grazing preference between *B. valdivianus* and *L. perenne* during winter, spring and summer. Whereas Chapter 6 assessed herbage mass production and nutritive value of *B. valdivianus* and *L. perenne* mixture under New Zealand rainfed conditions.

The last chapter discussed the main findings of the thesis and new hypothesis generated.

1.3 REFERENCES

Ewel, J. J. (1991). Yes, we got some bananas. *Conservation Biology, 5*, 423-425.

Chapman, D. (2016). Using Ecophysiology to Improve Farm Efficiency: Application in Temperate Dairy Grazing Systems. *Agriculture, 6*(2), 17.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture ,41*, 261-275.

Henessy, K., Fitzharris, B., Bates, N., Harvey, S., Howden, M., Hughes, L., Salinger J., & Warrick, R. (2007). Australia and New Zealand, climate change 2007: Impacts, adaptation and vulnerability. In M. Parry, O. Canzani, J. Palutikof, P. van der Linden, & C. Hanson (Eds.), *Contributions of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Hofer, D., Suter, M., Haughey E., Finn, J. A., Hoekstra, N. J., Buchmann,, N., Lüscher A., & Bennett, J. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology, 53,* 1023-1034.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science, 70,* 283-295.

Lee, J. M., Matthew, C., Thom, E. R., & Chapman, D. F. (2012). Perennial ryegrass breeding in New Zealand: A dairy industry perspective. *Crop and Pasture Science, 63*(2), 107-127.

Litherland, A., Woodward, S., Stevens, D., McDougal, D., Boom, C., Knight, T., & Lambert, M. (2002). Seasonal variations in pasture quality on New Zealand sheep and beef farms. In *Proceedings-New Zealand Society of Animal Production* (pp. 138-142). New Zealand Society of Animal Production.

López, I., Balocchi, O., Lailhacar, P., & Oyarzún, C. (1997) .Caracterización de sitios de crecimiento de seis especies pratenses nativas y naturalizadas del Dominio Húmedo de Chile. *Agro sur*, *25,* 62-80.

Parry, M., Canziani, O., Palutikof, J., van der Linden, P. J., & Hanson, C. E. (2007). Climate change 2007: impacts, adaptation and vulnerability Cambridge University Press Cambridge.

CHAPTER 2

Literature review

2.1 INTRODUCTION

The review examines the importance of pasture diversity in the production and persistence of the species with time, plant-animal interactions, and the knowledge gap to manage (mainly defoliation criterion) diverse pastures. Therefore, the objectives of the present review are to:

- Analyse how grasslands respond to plant diversity and its effects on the herbage production and persistence of species.
- Discuss the main morph-physiological changes of grass species due to competition between plants in a community.
- Discuss how grazing management influences species succession.
- Summarise the main environmental constraints that affect plant performance and relationships between species.
- Discuss animal selection in a mixed pasture and the consequences for long term succession of species.

2.2 THE GRASS FUNCTION IN AGRICULTURAL ECOSYSTEMS

Grasses dominate grasslands (i.e., given name to the ecosystem) and depending on how close or far from the Equator parallel the domination of C3 (cool-season grasses), C4 (warm-season grasses) or a codominance of both Calvin cycle species occurs. The structural characteristic of grasses allows them to resist grazing, fire, and extreme weather conditions (heat, cold). For many species, the growing points are near to the ground surface, which makes them unreachable for grazing animals during the vegetative phase. They are protected by immature leaves giving, among other characteristics, ecological advantages compared to other taxon to dominate agricultural systems (Leafe, 1988).

Native or natural grassland and anthropogenic pastures cover 31–43% of earth terrestrial habitats (Gibson & Newman, 2019). They are a refuge of a diverse fauna, and give ecosystem services, such as carbon sequestration, food supply and gene pool of many species (Gibson & Newman, 2019). Climate change is affecting the length and intensity of seasonal droughts (Overpeck, 2013), threatening species diversity on grasslands (Gibson & Newman, 2019). In New Zealand, warmer summers promote the invasion of undesirable sub-tropical species (e.g., *Paspalum dilatatum*, *Digitaria sanguinalis*, among others) with lower fodder quality than the main temperate pasture species (*L. perenne*) utilised in the pastoral farming system (Kenny et al., 2000). This species has been widely used in permanent cultivated pastures since 1930s (Charton & Stewart, 1999).

2.2.1 Perennial ryegrass (*Lolium perenne* L.)

Lolium perenne is native to Middle East, most of Europe and part of the Mediterranean (Balfourier et al., 2000). It has a fast establishment, produces well under different soil fertility conditions and contains an endophyte (*Epichloë festucae* var. *lolii*), a fungus which lives within it. The endophyte confers to the host protection against insect predators via production of alkaloids (Charton & Stewart, 1999). However, some endophyte alkaloids produced by *L. perenne* can be detrimental to livestock and can cause neuro-muscular (ryegrass staggers) and vascular constriction (heat stress) illnesses in cattle, sheep, deer and horses which can decrease animal production (Tor-Agbidye et al., 2001). *Lolium perenne* pastures without endophyte showed low persistence and production in New Zealand due to insect attack, predominantly the larva of Argentine stem weevil (Barker et al., 1984). Recently, thanks to the advance in research programmes, the development of novel strains of endophyte for *L. perenne*, such as AR1 and AR37, meet the criteria of plant protection without harming the livestock (Fletcher, 1999), and show a positive effect on animal production compared to wild endophyte (Bluett et al., 2005).

Lolium perenne based pasture production varied little in the last decades in New Zealand, with average pasture production of 10 t/ha in the 70s (Charles & Valentine, 1978; Smetham, 1990); while recently Chapman et al. (2015) reported an average of 12 t/ha for the first three years of pastures, and in a review article Lee et al. (2012) reported an average of 15 t/ha for top yielding cultivars under optimal growing conditions. McDonagh et al. (2016) reported an increase in dry matter yielding potential of 0.45% per year in the last 40 years in Ireland, which is close to the gain in dry matter yield potential in New Zealand (0.71%) (Harmer et al., 2016).

Pasture persistence was defined by Clark (2011) as 'maintenance of a desired species through time without major intervention'. Long pasture persistence is desirable because of the inherent cost of pasture reseeding and low production during establishment period (Wilkins & Humphreys, 2003). Pasture establishment is the most vulnerable period in the pasture life (García-Favre et al., 2017), and the risk of pasture establishment failure may increase due to climate change likelihood of drought or waterlogging. In a review paper, Clark (2011) assessed the main factors affecting perennial ryegrass persistence in New Zealand, being climate in some regions (i.e., summer drought and high winter rainfall), and intensive pasture management (i.e. low residual and late spring-summer mowing affecting seedling recruitment) the main contributions of a decreased persistence. However, a study in upper North Island (Waikato region) stated that abiotic (summer/autumn droughts) and biotic stress (damage by grass grub) were the main causes of plant loss of ~ 80% of perennial ryegrass in the pasture herbage mass in the autumn of the fourth year, and showing no differences among ryegrass cultivars and seeding rates (Lee et al., 2017).

Tetraploid cultivars of *L. perenne* compared to diploid cultivars, have greater nutritive value mainly explained by a greater dry matter digestibility (Balocchi & López, 2009).

However, the responses of tetraploid cultivars to abiotic stress differ. Tozer et al. (2017) noted there was an increase in shoot growth per unit of water added in tetraploid plants compared to diploid plants under limited soil moisture levels. Conversely, the tiller numbers were greater for diploid cultivars, which supports a faster recovery after drought conditions.

2.2.2 Pasture brome (*Bromus valdivianus* Phill.)

Many of the species from the *Bromus* genus are native to South America (Clayton & Renvoize, 1986). Pasture brome (*Bromus valdivianus*) is a native species from the South of Chile (Valdivia region), it dominates in high fertility soils and has a high herbage mass production under free drainage with medium texture soils (López et al., 1997).

Pasture brome showed greater herbage mass production and similar quality to *L. perenne* in an experiment in the South of Chile (Calvache et al., 2020). This greater production could be attributed to a better drought resistance as Keim et al. (2015) reported an increase in *B. valdivianus* and *D. glomerata* contribution with a decline in *L. perenne* in a three species mixture after the dry season. The better drought resistance in *B. valdivianus* could be partially explained by its deep root development as Stewart (1996) detailed for *Bromus* species in the *Ceratochloa* section, which enables water uptake from deeper soil horizons compared to *L. perenne* (Ordóñez et al., 2018). In general, this genus presents more and bigger sized leaves per tiller (Berone et al., 2007; Calvache et al., 2020), but less tillers per plant than *L. perenne* (Descalzi et al., 2018; López et al., 2013).

2.3 GRASS MORPHOLOGY AND PHYSIOLOGY AFFECTED BY ENVIRONMENTAL FACTORS

2.3.1 The effect of light on plant growth

Light is perceived by plant photoreceptors, such as phytochromes, cryptochromes and phototropin. Each of these receptors perceives different wavelength in the light spectrum, and stimulate morphological responses in the plants, called photomorphogenic responses (Ballaré & Casal, 2000). Tillering is a phenotypical character that is not only modified by intrinsic factors in the plant, such as phyllochron (defined as the time interval between the appearance of two consecutive leaves in a tiller (Skinner & Nelson, 1995), WSC level, leaf appearance rate, but also by a light signal. The low red/far red ratio (R/FR) reduces tillering and stimulates leaf sheath growth, which occurs when shading starts increasing in the pasture canopy at higher LAI (Gastal & Lemaire 2015). This condition is accompanied by a decrease in light interception per plant and lower carbon nutrition for tillering in high density swards (Casal, et al. 1986). However, FR light can trigger changes in grass tillering when the amount of biomass or the LAI are low, as showed by Casal et al. (1986) in an experiment with *Paspalum dilatatum*. These authors found that plants placed at low densities and a small degree of mutual shading showed a 40% reduction in tillering compared to isolated plants. The FR light is reflected by green plant parts and can impact on tillering responses during pasture establishment (Ballaré & Casal 2000). A small decrease in the R/FR ratio is enough to trigger morphological modifications, and during pasture establishment, it is an early indication of competition.

The light environment within plant canopies could be expected to differ between plant communities exposed only to intra specific competition and communities of plants with different architectures. Gatti et al. (2013) identified competition in the establishment period of two C3 grass species through changes in morphogenetic traits, but they did not assess modification in the canopy light environment between pure or mixed pastures that could have driven those adjustments. Although the R/FR ratio effect on growth has been well studied in grasses, little is known about the effects of neighbours with contrasting plant architectures in morphogenesis of two species coexisting in a grassland from the establishment period. Diverse swards could have an impact on plant morphogenesis from establishment compared to pure swards and with a carry-over effect on the future pasture performance.

The amount of photosynthetically active radiation (PAR) intercepted and the radiation use efficiency (RUE) by a pasture are closely related to its herbage mass production (Feldhake and Belesky, 2009). The New Zealand pastoral system aims to achieve a high growth rate with maximum efficiency of harvested forage between grazing events. First, Noy-Meir (1975) through the predator-prey model indicated the three phases of the growth rate of a pasture and the implications of managing the stocking rate to match animal intake with pasture growth. Later, Parsons et al. (1983) showed the importance of the net photosynthesis in the pasture and how the maximum value is achieved at 95% of light intercepted, where beyond this point there is a decline in the net biomass accumulation. Beyond this point the accumulation of dead material in the pasture outweighs the production of new tissue. However, this relationship between 95% of light interception, the decline of instantaneous growth rate and net photosynthesis, changes during stem elongation due to flowering (Parsons, 1988). During flowering the interception of above 95% of light does not mimic the reduction in growth rate and accumulation of senescent material. Although, if the grazing is later than this point it can cause problems with harvesting the forage by grazers (high amount of low-quality herbage material) and more tiller death due to a lack of light penetration at the bottom of the sward, and to the apical dominance in the plants as all the assimilates are allocated to seed production. Thus, as Parsons (1988) detailed a different grazing strategy should be pursued during stem elongation.

2.3.2 The effect of soil water content on plant growth

A stress in a plant was defined by Grime (1977) as any factor that limits herbage production. The factors that affect plant growth can be abiotic (moisture level, temperature, nutrients) or biotic, such as competition between species for resources or damage by herbivory. Soil water constraint is responsible for most of production losses in both natural and sown pastures in the world and will be a greater problem in the near future (Elliott et al., 2014).

Perennial grasses, in response to soil water constraint, have two main strategies: dehydration avoidance and dehydration tolerance (dehydration escape is associated with annual species) (Turner, 1986). They can combine many strategies within these two main strategies and how they utilise them needs to be studied in relation to the intensity of the soil water constraint (Serraj & Sinclair, 2002). The relationship between plant resource allocation to overcome the water scarcity period and the intensity of the drought is not considered in much research. A schematic representation of plant strategies to respond to different soil water contents and, thus, how growth is affected is shown in Volaire (2018) (Figure 2.1).

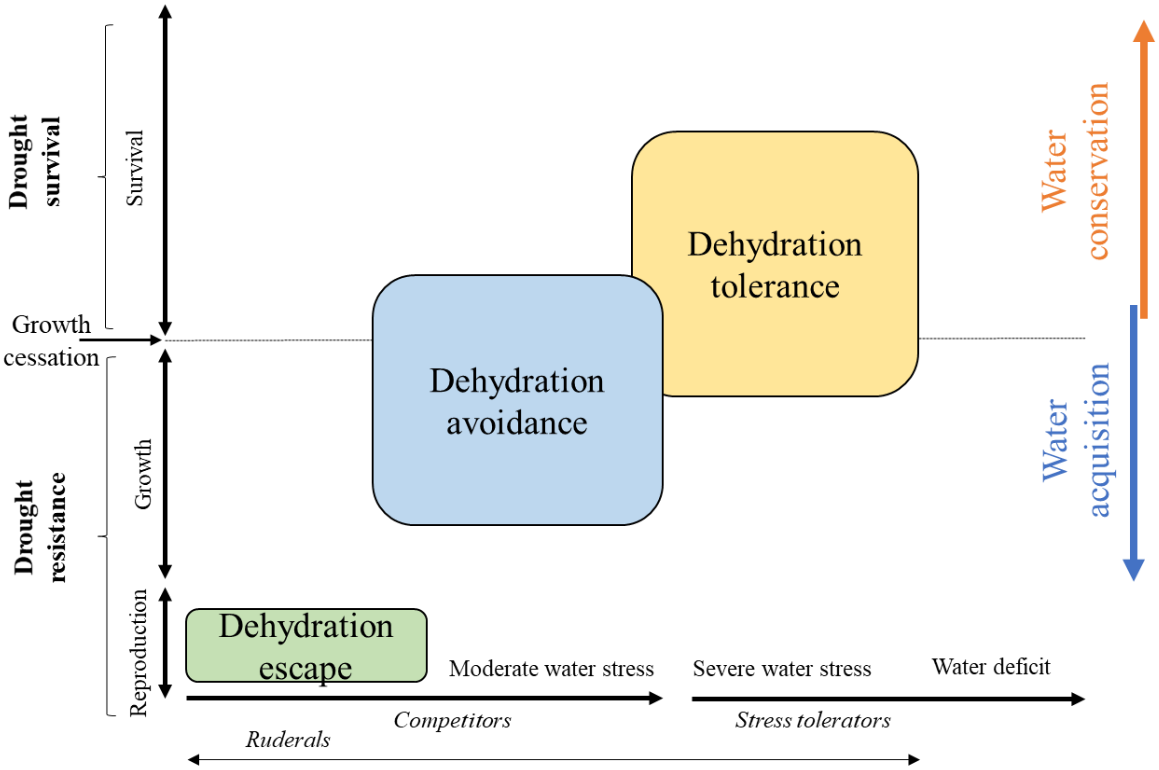


Figure 2.1. Relationship between increasing water stress intensity (x-axis) and growth stages of plants (y-axis), with the main strategies of the plants under the increasing water stress. Figure adapted from Volaire (2018).

The level and length of the drought period are important factors that trigger different physiological events in plants. Under moderate drought conditions, to maintain aerial growth, plants have to avoid and/or tolerate leaf dehydration. When drought is severe, plants response is associated with dehydration avoidance and tolerance, which are happening in the meristem organ (Volaire et al., 2014). Dehydration avoidance is associated with capturing a maximum of soil water and/or minimising water loss (Volaire, 2018). Root depth is a major trait of this strategy (Blum, 2009). On the other hand, plants with a tolerance strategy have tissues that are insensitive to dehydration (Volaire, 2018); thus they keep turgor in lamina tissues that allow maintenance of leaf area, leaf relative water content and photosynthetic capacity, and one of the most significant mechanistic traits that lets this happen is osmotic adjustment (OA). The OA (lowering of osmotic potential due to net solute accumulation) allows to harvest water from the soil retained at low potentials. However, OA was demonstrated as a poor strategy that enhances yield in plants with higher OA in leaves, as was shown by Serraj and Sinclair (2002) in an extensive literature review. High osmolyte concentration in the cytoplasm to maintain cell turgor does not seem an effective strategy when the soil water content is near permanent wilting point, as plants will keep transpiring and losing water with the possible outcome of death. In addition, keeping cell turgor will prevent not only stomata closure but also leaf wilting (Serraj & Sinclair, 2002). Nevertheless, Blum (2017) stated that Serraj and Sinclair (2002) OA roles hypothesis was not appropriate, as OA in much research showed benefit in herbage mass production, because it is the main regulator of stomatal conductance and leaf turgor. However, they detailed the importance of a slow gradient of water loss in the soil to enable the plant for OA, as it is an adaptive process. This last statement exhibits the value of knowing the level of stress in the plant, the soil water content at permanent wilting point, the energy to which the water is retained by the soil, and lastly the duration of the water constraint. The OA could be important at low water deficit or short-term drought, but it seems difficult for plants to maintain growth as water deficit intensifies (Barker & Caradus, 2001). Most of the research in this area has a lack of information regarding these last factors.

Net photosynthesis of a plant does not respond equally to growth processes under moderate water shortage in the soil as the former is less sensitive to resource limitation (Korner, 1991). Under this circumstance, carbohydrates accumulation in storage organs is surplus to growth requirements. Eventually, if the soil water shortage becomes severe, net photosynthesis and growth are restricted and plants allocate resources to maintain primary metabolic processes and respiration (Herms & Mattson, 1992). These physiological changes cope with soil water availabilities and affect dry matter accumulation and pasture survival during and after the abiotic stress. Therefore, pasture production and survival could be aggravated if pasture management under the abiotic stress is not closely related to the pasture physiology strategy (e.g., less plant survival).

Pasture defoliation management under low water status in the soil is crucial for sustainable production. However, in the literature, research looking for the best defoliation practice that is associated with plant traits to overcome water deficit period are sparse.

2.4 PASTURE DEFOLIATION MANAGEMENT AND ITS IMPACTS ON PLANT COMPETITION

During the 1980’s–90’s, research focused on the effect of grazing on grasslands at the level of species survival. Some research suggested a low species survival when above ground biomass is defoliated by large mammals was a result of a decline in root reserve (Belsky, 1986). However, there is much evidence supporting that herbivores do not cause any damage to root growth (McNaughton et al., 1998), but in fact improves net aerial biomass production (McNaughton, 1979), by removing senescent material and enhancing nutrient cycling (McNaughton et al., 1989). The research conducted by McNaughton (1979) and McNaughton et al. (1998) were in Serengeti, Africa, where grasslands are grazed using a high stocking density of mammals over a short period of time, and plants have a long period of time between two consecutive grazing. Severe defoliation (i.e., high intensity), and high defoliation frequency decrease perennial grasses survival in grasslands, which is exacerbated by increased competition from invasive species and unpalatable species that benefit from heavy grazing (i.e. more grazing pressure on desirable species) (O'connor, 1991).

Plants respond differently to grazing, and there are two main strategies behind the responses: tolerance and avoidance of grazing. The former, is an important strategy between forage grasses and depends on the interaction of intrinsic (genetically) and extrinsic factors (influenced by the environment). Intrinsic factors correspond to physiological traits such as growth rate, storage capacity of compounds (such as WSC in stubble), allocation patterns and the flexibility of photosynthetic rates (Rosenthal & Kotanen, 1994). Some morphological traits like morphological plasticity, which together with soil resources available for plants and competition between species for those resources, are critical for the grazing tolerance of species (Maschinski & Whitham, 1989).

In a mixed pasture, competition among species or genotypes is defoliation management (frequency and intensity) dependent (Hazard & Ghesquière, 1995). An adequate defoliation management prevents the excessive dominance of one species over another desirable species (Peltzer & Wilson, 2000). Thus, in order to achieve coexistence of two species and enhance their equilibrium in a pasture it is necessary to reduce competition from the most dominant ones, which is possible by selecting appropriate grazing management (Tow & Lazenby, 2000). Many models have been discussed to assess management of grasslands. In order to seek positive improvement, the state-and-transition model formulation to rangeland management considers grazing as a tool that should not be fixed and should be adjusted to different environmental conditions (e.g. drought, flood), the model pursues an increase in the presence of high growth rate species in the vegetation community (Westoby et al., 1989). Manipulation of the stocking rate and grazing pressure control animal intake and their ability to select among species and organs of the plants, and thus it affects the pasture botanical composition by allowing for a greater or lesser selection of palatable species (Kemp & King, 2001). Related to defoliation management in a mixed pasture, the defoliation frequency has been reported as an important factor that alters species growth dynamics (Gatti et al., 2017). The differences among high, slow, and intermediate defoliation frequencies can cause a wide variation of morpho-physiological changes in the species (such as tillering, leaf size), due to phenotypic plasticity. However, these modifications depend on the species, which organs are involved and how intensely they are defoliated (Harris et al., 1981).

In the last two decades, much progress has been made on understanding the effects of defoliation management on pastures, with a comprehension of the importance of grazing frequency taking into account plant-related indicators; which allows the maximisation of both production and persistence of plants through time. Thus, regarding defoliation management, the leaf stage (LS) concept constitutes a key parameter based on the number of leaves at individual tiller level, which assures a replenishment of WSC in the plant and a fast regrowth after defoliation until the photosynthetic tissue is capable of net carbon assimilation (Fulkerson & Donaghy, 2001). Grazing beyond the optimal range stage will lead to senescent material and lower forage quality. There is a minimum number of leaves/tiller for which grazing before that stage is achieved reduces plant capacity to survive in stressful conditions (low soil water content, extreme temperatures), as insufficient WSC will be replenished for the next regrowth phase. Therefore, plant indicators as a criterion to manage grazing defoliation are WSC replenishment and start of senescence of leaves.

Perennial ryegrass (*L. perenne*) optimum leaf stage range for the start of grazing is 2.5 – 3.0 (Fulkerson & Donaghy, 2001). Whereas, for *B. valdivianus* it is 3.5 – 4.0 LS, but in the case of *B. valdivianus* it is still necessary to determine the pattern of WSC recovery for the suggested leaf stage range (Ordoñez et al., 2017). These two species are mentioned as an example, but several pasture species can be used as part of a pasture mixture, such as *Festuca arundinacea* or *Dactylis glomerata*. These species differ in LS because their life span and number of leaves per tiller are dissimilar (Donaghy et al., 2008; Turner et al., 2006a). This difference in LS occurs with most grass species, and so the LS of two species in a mixture is unlikely to match, as is the time needed to reach the suitable LS for defoliation, as the phyllochron among the species are usually different. Therefore, in order to manage the pasture appropriately (enhancing production and survival of its individual plants), it is important to assess the degree of overlapping of LS between two species in a mixture.

To date, research has been focused on competition between species in mixtures or comparing the performance of monocultures against mixtures in order to identify any advantage of the second (Husse et al., 2016). Nevertheless, little has been researched on species defoliation at optimal leaf stage to ensure productivity and persistence of a mixed pasture. Defoliation criteria have been based on pasture height, herbage mass or on a defoliation frequency based on a fixed number of days. Pasture height is an indicator that does not reflect pasture density, so the horizontal structure of the pasture is not considered. Pasture mass is more precise, but could lead to defoliation of a pasture with high senescent biomass; for example when environmental constraints impede achievement of the defoliation target to satisfy animals demand and then when this moment is achieved plants have dead leaves with an overall poor quality in the pasture (Fulkerson & Donaghy, 2001). Turner et al. (2006b) reported better productions in three grass pastures when gazing interval was near leaf stage, before the start of senesce of the first leaf. Replenishment of WSC is vital for regrowth after grazing and the most influential factor that affects WSC is the defoliation frequency. Increasing frequency of defoliation over spring, below optimal leaf stage, had severe implications on the survival of the plants over summer: it increased tiller mortality in a subtropical environment (Fulkerson & Donaghy, 2001). Kaufononga et al. (2017) in a trial in New Zealand reported a reduction in tiller density when *L. perenne* pasture was defoliated below three leaf stage. Yet, there is no information in the literature about how to manage a diverse pasture, as much of the research regarding pasture defoliation management based on leaf stage has been made in monoculture pastures and clover and grass mixes (except for Gatti et al. (2017)). In addition, species selection to compose a pasture (mainly from seed companies) are made for biomass production in monospecific swards without competition from other species or under different grazing managements (Hoveland, 1997).

Lolium perenne is very competitive when grown in companion with other grasses in cold/temperate environments (14 °C day/8 °C night) regardless of the cutting frequency, however, when day/night temperature were higher (24 °C day/18 °C night) domination by C4 species (*P. dilatatum*) occurred, but only under infrequent cutting (Harris et al., 1981). If animals interact with the pasture through diet selection, then they modify how different species interact. López et al. (2016) stated that dairy cows have selected *B. valdivianus* over *L. perenne* when they have grazed a mixed pasture and the morphological characters that defined selection criteria changed among different seasons. Nevertheless, long term defoliation management induced a type of vegetation structure (tillers size and numbers) that modifies competitive interactions between species, such as changes in the composition of the pasture made by selective defoliation of plants (Briske & Hendrickson, 1998).

2.5 RELATIONSHIP BETWEEN FUNCTIONAL DIVERSITY AND PASTURE PERFORMANCE

In a climate changing world with increasing temperature and rainfall variability between years, enhancing plant diversity is one strategy to maintain and improve annual herbage production in pastures (Hooper et al., 2005), due to interspecific interactions (Finn et al., 2018). These interactions among plant species and their habitats are based on the functional diversity, as defined by Tilman (2001) as “those components of biodiversity that influence how an ecosystem operates or functions”. Plants have many different traits (i.e., nitrogen fixation, root architecture, life habit, allelopathy, among others) so it could be thought that increasing the number of species in a pasture will result in more stable herbage production than a binary mix. However, Tilman et al. (1997) demonstrated that functional diversity generated more productivity compared with species diversity alone. Moreover, they stated that the addition or disappearance of some species can have a major impact on an ecosystem; however, the degree of the impact changes among species due to the different functional traits that they hold. Thus, in a pasture mixture, species with different functional traits produce the so-called "mixture effect" that enables a greater sustained production of biomass compared to monoculture pastures at the same level of available resources; as long as there is a considerable micro variation in resources in the environment where different species can explore them (Harris et al., 1981; Sanderson et al., 2004). The degree to which a mixture capture resources is one of the determinants of the superiority (Roscher et al., 2011).

Light interception is an important factor to achieve high amounts of biomass production (Brougham, 1956), but higher herbage mass production in a mixed pasture than the monocultures of its component species it is not based on the improvement of light interception for high productivity conditions. Husse et al. (2016) reported that asynchrony in shoot growth among species is the key factor that drives the so called “mixture effect”. Such increase in annual herbage production is a major contribution to greater sustainable production, as higher production can be achieved from the same level of resources (Foley et al., 2011). Mixing four species with contrasting season growth (*L. perenne, T. repens, Trifolium pratense* L. and *Cichorium intybus* L.) increased herbage mass 23% over four years evaluation compared to its monocultures due to asynchrony in shoot growth (Husse et al., 2016). Turnover of labile organic matter is affected by differences in litter quality and timing of litter deposition between plant species; and metabolic components of plants contribute more significantly than structural components. In diverse pastures different plants phenology, and timing of organ senescence could enable an increase in overall decomposition or nutrient turnover (Vitousek & Hooper, 1994).

The effect of increasing diversity in production started with the replacement series studies (varying the proportion of two species) (De Wit, 1960). The assessment of increasing diversity with species that hold different functional traits started with Donald (Donald, 1963), who wondered if the increase in the number of species and the complexity in a pasture enabled the community to exploit the maximum resources. Further, Donald (1963) pointed out the possibility to enhance water and nutrient capture by different root types (tap, fibrous, etc) among species. He assessed that the relationship between two species within a pasture could lead to different results in the performance of each one, as this depended on the level of supply of the factors for growth, and the niche of each species: a concept that indicates the function that a species occupies in a community; two or more species with identical niches could not coexist as one would displace the other (Gause, 2003). Thus, it is important for species coexistence that the individual species explore different niches (e.g. perennial ryegrass and white clover) within the habitat (Hooper et al., 2005).

Root development is part of the niche exploration, and within a plant community the existence of species which differ in root architecture not only improves the above ground biomass production but also community survival due to facilitation between species to face environmental constraints (Larcher, 2003; Sekiya et al., 2010). Complementarity (greater use of limiting resources) or facilitation (e.g. mutualism) depends on the different functional traits among the species growing in the pasture; thus these processes can be maximised when the species differ in spatial distribution, growth pattern, or amount of resource capture (Hooper et al., 2005). ‘Overyielding’ is a consequence of greater production in mixtures compared to monoculture yields, and complementarity/facilitation are the two primary mechanisms that allow overyielding (Loreau, 1998). If the mixture produces more than the best monoculture yields, the overyielding is transitive; whereas if it does not exceed the yield of the most productive monoculture it is non-transitive (Hooper & Dukes, 2003). In a four year study in Australia, it was reported a low persistence (close to 50%) of mixtures with shallow root species (ryegrass and white clover), while mixtures with deep root species (*F. arundinacea* and *D. glomerata*) showed ~100% persistence (Nie et al., 2004). However, this study did not evaluate a mixture of shallow and deep root species to assess facilitation and competition process between them, and therefore the implication of these processes on the persistence of species in this type of mixture.

It is possible that a lower yield of mixtures compared with monocultures is a consequence of competition for limited resources (Sackville-Hamilton, 2001). In plant communities, competition may not be avoided as plants share some niches along with their existence. McNaughton and Wolf (1970) defined domination as the annexation by dominant species of the potential niche space of subordinate species, and they stated the importance to separate plant niche parameters in order to understand the relative dominance of species in a community. In some cases, the fitness of plant species to the environment implies that one species is stronger than others, which leads to total suppression of its neighbours and to a higher growth rate as the final result of the competition-domination process (Frame, 1973). McGilchrist and Trenbath (1971) emphasised that in a mixed pasture there is a dominant species, and each individual plant of this species get greater herbage mass production than plants in its monoculture (i.e., this study assessed the mixture of three varieties of wheat). Conversely, the dominated species shows a decrease in yield in relation to its own monoculture.

Most of the research around pastoral species diversity has focused on the relationship of the species above the ground level and how they interact with each other in relation to the herbage production, and the coverage of the space (Tejera et al., 2016; Vojtech et al., 2008). Less research has focused on species relationships below ground and how this may affect above ground herbage production. Seager et al. (1992), in hill country in Wanganui, New Zealand, reported that the effect of root competition from established pasture plants on seedling growth of transplanted perennial ryegrass was greater than the effect of shoot competition and similar to full competition (i.e., root and shoot competition between plants). Competition below ground is more significant when the availability of abiotic resources fluctuates through the seasons.

Wilson (1988) and then, in a meta-analysis, Kiaer et al. (2013) concluded that root competition was greater than shoot competition for resources under low nutrient levels. Conversely, shoot competition was more important at higher nutrient supply levels, with shading as the main factor affecting plant growth; which has implications for the dominant and dominated plant nitrogen acquisition (Kiaer et al., 2013). Dominated plants have less access to light and become less able to absorb nitrogen from the soil, and a consequence of this is that they cannot maximise leaf expansion for light capture (Lemaire, 2001). Moreover, the dominance of some species over others can be a result of a differential response to climate variation between seasons or a consequence of grazing selectivity or simply different growth habits (Tow & Lazenby, 2000). Environmental factors, such as climatic and edaphic, can modify the expression of morphological and physiological plant traits that determine competitive relations between species (Nurjaya & Tow, 2001).

How plants interact within a community and within their habitat depends on their morpho-physiological strategies and how these strategies interact with the surrounding environment. Grime (1977) proposed the competition-stress-disturbance (CSD) model, which states three main life strategies that separate species depending on their phenotypical response to disturbance, stress and competition. Competitive (C), stress-tolerant (S) and ruderal (R) (adaptive to frequently disturbed areas) constitute the main species survival and colonisation strategies. There are secondary strategies that place plants in some point between main strategies, as plants carry out the combination of two or three main strategies (Figure 2.2). The strategy of each species has been defined based on measured attributes, such as growth rate, leaf extension, specific leaf area, leaf nitrogen content and palatability. Some researchers have tried to mix species in a pasture with similar C-S-R strategies with the aim to measure the outcome of competition between them. For example, Tejera et al. (2016) evaluated the competition among one cool-season and two warm-season perennial grasses, and they found that inclusion of a warm-season species negatively influenced the performance of the dominated species in the mixture. Nevertheless, the degree of competition with the cool-season species depended on the type of growth habit of each warm-season species, showing the importance of species morpho-physiological characters in the relationship between species in a plant community.

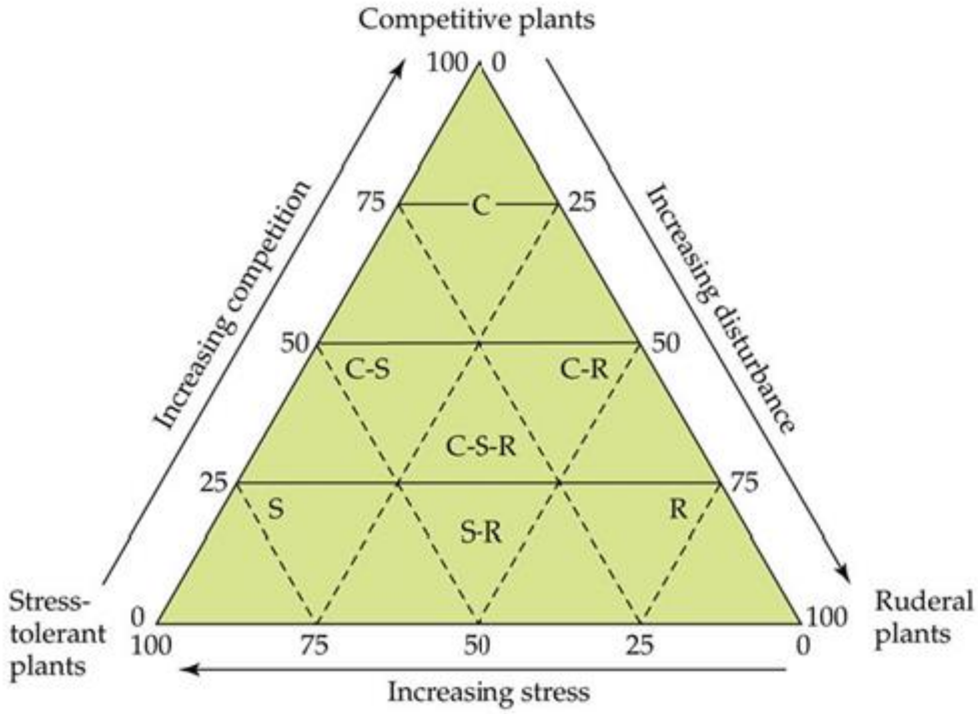


Figure 2.2. The competition-stress-disturbance model that shows the three primary strategies of plants and where a single species can be found in the triangle. Adapted from Grime (1977).

In the literature it is difficult to find research showing a higher herbage production in a mixture of perennial grasses compared with their monocultures, as most of the research evaluated mixtures that sustained greater yield has a focus on the presence of a tall deep-rooted herb species (mainly under drought), such as chicory or plantain (Husse et al., 2016; Marquard et al., 2009). An explanation of this is that the habitat, where the species are not native or not adapted, has to offer resources so that these species can compete more effectively than the current inhabitants (McNaughton, 1977), something that is not pursued in most of the studies. However, beneficial relationships between species that differ in Grimes' strategies can be possible to obtain. Graff and Aguiar (2017) reported facilitation between stress tolerant species and competitive strategy species in a semi-arid region when both types of species coexisted in an environment without severe resource stress. A clear understanding of the competition process is relevant to comprehend species succession (changes in botanical composition) in pastures and from there to determine management practices to enhance long-term sward performance, with an understanding of soil-plant-animal relationship (Tow & Lazenby, 2000).

Tow and Lazenby (2000) emphasised the progress that has been made by many authors on understanding competition in a community, but not on the appropriate grazing management practices (defoliation frequency and intensity) for that community. Moreover, Grime (2002) reviewed a number of studies targeting the effect of diversity on productivity: quantifying yield in mixtures compared with monocultures and the additive effects of the number and type of species. It was concluded that species number and type are much less important than the influence of environment and grazing management on the pasture's performance. For example, fixed interval mower outcomes for different species succession compared with pastures defoliation by animals, as it is highly probable that in response to animal selection for palatable species the relationship between species in the mixture changes (Duru et al., 2014). Although, species diversity and the use of different niches in a plant community stabilise community functional properties to face environmental disturbances (McNaughton, 1977), showing the importance of species diversity in a pasture despite greater complexity in pasture management.

2.6 MORPHO-PHYSIOLOGICAL RESPONSE OF PLANTS TO DEFOLIATION REGIME

Obtaining high efficiencies of primary production in pastures is a consequence of the interaction among different factors including climatic variables (rainfall, temperature, photoperiod), and grazing management (defoliation intensity and frequency) on pastures. These grazing factors interact with the climatic factors mentioned and have a direct effect on the productivity and persistence of the species within a community. Light is an important resource for plant growth, but its capture and transformation into carbon forms is highly inefficient. From the total photosynthetically active radiation (PAR) that the canopy receives, less than 1% is converted into dry matter and approximately 0.02% of the PAR becomes secondary production (Nabinger, 1998). This evidences that appropriate agricultural practices are necessary to guarantee the maximum energy utilisation efficiency of the system, of the energy captured by plants. In a pastoral ecosystem, defoliation is a central factor to achieve this, where the optimal defoliation moment is related to the maximisation of the average pasture growth rate, to a high-accumulated biomass production and to a senescence rate that does not reach its maximum (further from this stage the senescence matches the net growth rate) (Parsons & Penning, 1988). The defoliation in this “optimal moment” is closely related to the leaf area index (LAI, i.e., ratio between leaf area per unit of ground area) intercepting 95% of the incident radiation, called optimal or critical LAI. The value of optimal LAI and the time that a pasture takes to reach it depends on physiological and morphological characteristics of the pasture, which interact with the environment and pasture management (Chapman & Lemaire, 1993; Gastal & Lemaire, 2015). However, the optimal LAI, the moment when a pasture should be defoliated according to its LAI, is complicated to perceive without a visible plant morphological indicator. Defoliation frequency based on leaf regrowth stage (LS; number of fully expanded leaves per tiller) is a compromise between pasture growth physiology and pasture utilisation, which allows optimising pasture yield, post defoliation pasture recovery and persistence (Fulkerson & Donaghy, 2001). The pasture growth during the first days post defoliation is strongly related to the amount and utilisation of the water-soluble carbohydrate (WSC) reserves. The replenishment of WSC in the stubble of plants during the regrowth period after defoliation is what determines a LS range, in which a pasture should be defoliated (Donaghy & Fulkerson, 1998).

In production systems, both ontogenetic and phenotypic plasticity expression in pasture grasses usually functions as part of an environmental sensitivity of the plants to face changes in the soil water content and environment temperatures between seasons (Wright & Mcconnaughay, 2002). Phyllochron is an ontogeny event that could be modified by the environment (e.g. soil water constraints) (Wilhelm & McMaster, 1995); it is temperature dependent, and alters tiller production (Nelson, 2000). In addition, phyllochron is modified by leaf elongation rate (LER) and sheath length that composed the whorl where the leaf is growing (Durand et al., 1999; Insua et al., 2018; Skinner & Nelson, 1995). Instead, phenotypic plasticity is not an ontogeny change and is related to changes in size, structure and spatial positioning of organs, what is known as architectural and morphology adaptation, due to environmental effects and defoliation management (Huber et al., 1999). Thus, the development of species through the appearance of new phytomers depends on the phylogenetic history of the species, the ontogenetic stage and environmental conditions (Huber et al., 1999). For a better understanding, Chapman and Lemaire (1993) conceptualised the different factors affecting regrowth after defoliation and how they interact between them (Figure 2.3). Leaf elongation rate, leaf appearance rate and leaf life span are morphogenetic characters that build up structural characters (leaf size, tiller density and leaves number per tiller) that are responsible for the yield outcome.

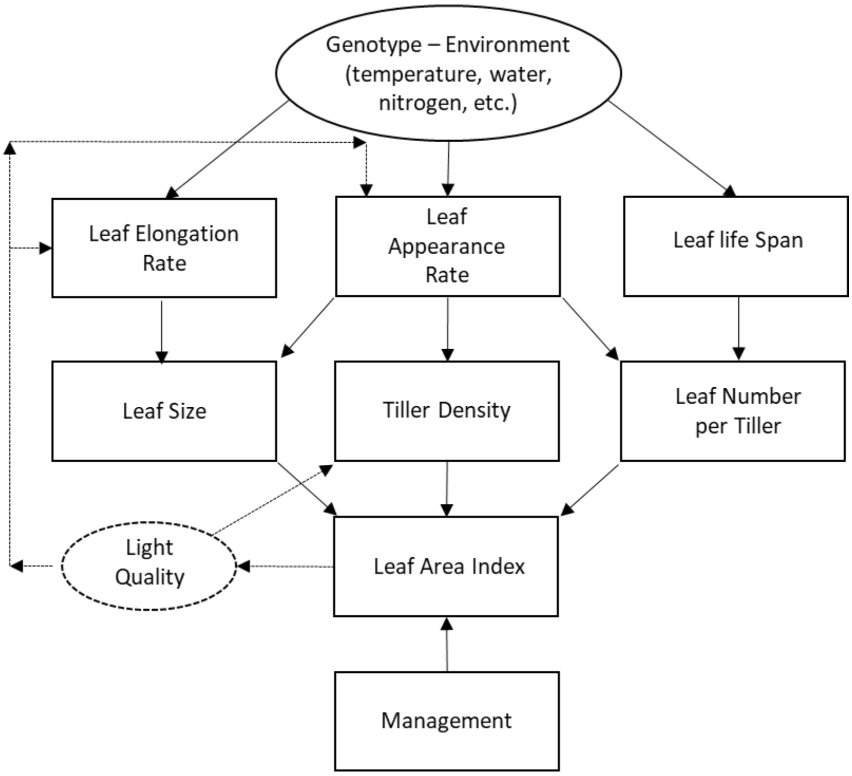


Figure 2.3. Model adapted from Chapman and Lemaire (1993) that summarises the relationship between plant characters.

The effect of temperature on plants is related to physiological changes, in general by the actions of enzymatic activities (Bonhomme, 2000). Temperature is the main driver of development processes in plants and plant development can be accelerated with increasing temperatures (Badeck et al., 2004). Thermal time (degree-day unit) is a measure of time in the form of accumulated growing degree-day (AGDD) and for temperate species is an accurate way to identify ontogenetic changes in the plant and was defined by McMaster and Wilhelm (1997) as follows:

$$GDD = \left[\frac{(T_{max} + T_{min})}{2}\right] - T_{base}$$

where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum temperature and T_{base} is the temperature below which the process of interest does not progress.

Even though phyllochron is related to thermal time due to its high dependence on temperature, it can also be modified by other environmental constraints, such as, soil water availability (Bartholomew & Williams, 2006). *Lolium multiflorum* (Italian ryegrass) increased the phyllochron when soil moisture content was below 15%, but when soil moisture was 30 or 45% it was not modified. Although, the delay in plant development could be a consequence of plants getting near permanent wilting point, as leaf appearance is decreased because of the reduction in the division zone length of the leaves (Durand et al., 1999). Cell division in a leaf is negatively affected by water stress in the plant, decreasing leaf elongation rate (Durand et al., 1995). Structural characteristics of leaves (sheath length) also can increase or decrease the phyllochron (Fournier et al., 2005). A reduction in the phyllochron is related to a decrease in the elongation zone and a lower flux of cells within the leaf when the whorl is shorter than a plant under optimal growth conditions. Fournier et al. (2005), assessing *F. arundinacea* leaf growth, showed that the shortness of the whorl could be a consequence of temperature being lower than optimum for leaf growing or defoliation below the ligule in a leaf, and demonstrated that thermal time and phyllochron time were not equivalent across treatments or seasons. Therefore, it has been shown that phyllochron changes with the intensity of defoliation, however how the phyllochron is affected by the interaction of different defoliation frequencies and soil water restriction is still unknown. This has a major implication for varying the timing of defoliation through a delay or not according to the optimal LS range.

Grasses adapt their growth, tillering, and leaf size to gain resources under conditions of stress or defoliation management practices. In order to assess the strategy of the species in terms of morpho-physiological adaptation, it is important to define which plant character is affected and, as a result of resource depletion or competition with other species, assess the morpho-physiological change in the traits with variable environmental resources. Within a community of grasses, tiller dynamics (tiller birth and death rate) of each species changes seasonally (Matthew et al., 2013), and is an accurate variable to determine pasture botanical composition and the effect of competition among species. In perennial grasslands, lateral proliferation of tillers is a mechanism of invasion of one species to another (Silvertown et al., 1994). Teughels et al. (1995) evaluated the competition between *L. perenne* and *F. arundinacea* when sown in a 1:1 mixture and found that *L. perenne* suppressed *F. arundinacea* growth through a greater leaf area production per plant. *Lolium perenne* was capable of sequestering more CO_2 and capturing more light, allowing for faster recovery after defoliation compared with *F. arundinacea* under high growing conditions. In a glasshouse study, *L. perenne* and *B. valdivianus* utilised different growth strategies that let them survive under environmental stress and competition (López et al., 2013). *Bromus valdivianus* produced larger and deeper roots per tiller than *L. perenne* under different soil moisture conditions. A higher concentration of roots in *L. perenne* is located in the upper 10 cm of the soil (Ordóñez et al., 2018). *Lolium perenne* strategy, regardless of the soil water restriction, was a lower allocation of resources to roots but not to tillers, producing a larger number of small tillers per plant that resulted in the same total shoot weight and root weight as *B. valdivianus*. There are two structural strategies within grasses in relation to survival through time that could be complementary: plants with a large number of tillers or alternatively larger tiller size. In temperate perennial grasses, a high tiller density is desirable to tolerate grazing and is a superior strategy for growth where hard grazing prevents the development of large leaves or tillers (Kemp & Culvenor, 2010). The effect of defoliation intensity and frequency on tiller density has been widely reported (e.g. (Briske & Stuth, 1982; Matthew et al., 1995)), as has the modification of morphological components of a tiller (Gastal & Lemaire, 2015). However, how defoliation frequency is best managed according to a leaf stage criterion in mixed pastures and how it modifies species competition over the time have been little addressed.

After a defoliation event, Nurjaya and Tow (2001) emphasised that the competitive ability of species in a mixture is a consequence of traits such as LAI, specific leaf area (SLA) and amount of WSC accumulated and used. These features allow some species to capture more resources such as light, water and nutrients. When the performance of *L. perenne* with increasing plant diversity in the community was evaluated, Roscher et al. (2011) found *L. perenne* plant numbers decreased due to a reduction in resource acquisition, light in this case. The result showed that there was no beneficial effect of complementarity in the use of resources when increasing the diversity of species in a community and showed poor performance when *L. perenne* was mixed with other grass compared to legumes. Although, the plots used in the experiment of Roscher et al. (2011) were mowed twice a year and therefore could have negatively affected the development of the species, as *L. perenne* vegetative growth is stimulated by frequent cuttings (Fulkerson & Donaghy, 2001). Many of the plant competition studies have been conducted on resources, such as light and nutrients, regardless of defoliation management. Defoliation management based on LS of plants and its effects on relations among different species in a plant community has not been tested. Similarly, traits relevant to resources use and survival capacity of species have not been linked with an optimal LS range for a particular community of two or more species; and not even how managing mixed pastures according to a LS criterion benefits coexistence between species.

Research to enhance pasture production under rain feed conditions in New Zealand observed a better yield *F. arundinacea* in summer compared to perennial ryegrass, across all LS used in the experiment (one, two and three LS for perennial ryegrass; and one, two and four LS for tall fescue) (Kaufononga et al., 2017). However, tall fescue pasture defoliated at four LS (i.e., showed higher fescue persistence compared to lower LS) only maintained ~ 50% of its plant density two years after sowing, which indicated its poor persistence and a future problem to maintain high yields. In addition, *F. arundinacea* at optimum defoliation interval (four leaves) had a low ME (9.5 MJ/kg DM) for adequate animal production. Under drylands conditions in southern Australia, Turner et al. (2006b) compared three grass monocultures, each defoliated according to its optimal leaf stage. They reported a better yield of *Bromus willdenowii* K. (prairie grass) than *L. perenne* and *Dactylis glomerata* L. (cocksfoot), but the best quality was obtained by the latter; however, they did not evaluate a mixture treatment to measure how species could complement each other.

2.7 GRAZING BEHAVIOUR AND ANIMAL SELECTION

Plants strategies to avoid herbivory need not to be solely related to herbivory repellence, but the need to be less desirable than neighbouring plants (Launchbaugh, 1996). Herbivores can recognise plants differing in nutritional attributes, such as nutrients and energy (Forbes & Kyriazakis, 1995). Thus, if they have the opportunity to choose between species and even between plant parts that differ in the nutritive value, they will perform a positive selection (Cave et al., 2015). Thus, grazers can learn to prefer or avoid foods based on metabolic effects when they previously tasted them and developed what is called ‘nutritional wisdom’. Apart from the nutrient value of the food, the physiological requirements of the animals are also important in the diet selection (Forbes & Kyriazakis, 1995).

Intake rate is the product of bite rate, bite mass and feeding time (Allden & McD. Whittaker, 1970). Animal performance is a complex trade off among the last three parameters, with a reciprocal relationship between bite mass and bite rate. Changes in bite mass will not lead directly to changes in intake rate as bite mass increases, bite rate decreases (i.e., time per bite increases) (Allden & McDWhittaker, 1970; Penning et al., 1991). The bite scale is at the bottom of foraging hierarchy, biting is one of the first actions in the grazing process, and any error animals make in selecting bite will be aggravated over the time (de Faccio Carvalho, 2013). The bite mass is the most important factor in the intake equation (as bite rate is modified by bite mass), and it is closely related with the pasture height, with a linear relationship between the them (Edwards et al., 1995).

Herbivore consumption of more palatable species in a mixed pasture encourages the success and persistence of less palatable species by impairing competition mechanisms of the former (Herms & Mattson, 1992). However, as Briske and Hendrickson (1998) showed in a semiarid savanna grassland in United States, selective grazing does not always trigger a domination of the non-defoliated species through the effect on interspecific competitive interaction. In this case, the minimisation of herbivory effect could be a consequence of the occurrence of a transitional stage in the vegetation community and/or the short temporal scale of the study diminishing the effect of seasons and years on interspecific competition (Briske & Hendrickson, 1998). Conversely, long term studies (45 years), in a Juniperus-Quercus savanna, showed the influence of different grazing intensity on the vegetation dynamic and the importance to evaluate environmental conditions, as the grazing factor significantly iterated with the environmental constraints to determine the vegetation community (Fuhlendorf & Smeins, 1997).

Secondary metabolism in plants is believed to have a major role deterring herbivory (Hernández-Agramonte et al., 2018). Alkaloids produced by grass infected with epichloid fungi is one important example of the mechanisms to herbivore resistance (Vicari & Bazely, 1993). This grazing resistance in grasses is known as an avoidance strategy, while a plant that grows fast after a defoliation event is associated with a tolerance strategy (Stuth, 1991). Fast growth species in a rich environment are less resistant to grazing but they are the most tolerant species to herbivory, as they rapidly replace photosynthetic tissue after defoliation (Briske et al., 1996). However, allocation priorities to leaf growth after defoliation (associated with a tolerant species) and low abundance of that species in the community can be a signal of high animal selection for that species (Briske et al., 1996). Thus, if the research objective is to study species succession in mixed pasture, it is necessary to utilise grazers and obtain a plant-animal interaction.

2.8 PREFERENTIAL GRAZING AS PART OF ANIMAL BEHAVIOUR

Preferential grazing was described by Allen et al. (2011) as “a measure of relative intake of alternative forage or forages constituents, where access to forage is unrestricted”. In other terms it is the discrimination exerted by animals given the minimum of physical and environmental restrictions (Hodgson, 1979). Thus, the theory states that when animals are given free choice between two fodders, they may show a preference for one over the other. What animals select is influenced by three variables with the importance: the animal, the environment where they are kept (i.e., environment homogeneity) and the characteristics of the forage that is being offered (Emmans, 2007). Nutritional factors can influence diet selection among sheep, as was shown by Kyriazakis and Oldham (1993) where sheep selected a diet that ensured their protein requirement for maintenance and growth. However, during the day, sheep showed a diurnal pattern of preference selecting legumes in the morning, but increasing the proportion of grass in the diet in the course of the afternoon (Parsons et al., 1994). This shows that sheep eat mixed diets and there are several theories explaining the cause of that behaviour (see Rutter (2006) for a broad explanation of each theory). Briefly, it is possible that animals eat a mixed diet and behave with a diurnal pattern for many reasons (and not just one), such as weather conditions (possibility of heat stress during the day), anti-predator behaviour, nutrient balance, maintaining a healthy rumen, among others (Rutter 2006). Thus, in each study it is important to define the conditions limiting animal intake and then animal preference.

In a preference study with sheep, Parsons et al. (1994) found that previous diet offered to sheep had a lasting effect on preference. In addition, they remarked that animals grazing a monoculture changed their preference in the first days when offered a mixture pasture. However, after some days, they reverted to a preference for the diet supplied before, but the explanation was unclear. A small reduction of species biomass at the beginning of the experiment could lead, also, to a higher growth of the other species and structural changes in both species; and herbage mass is one of the most important variables in diet preference, in companion with crude protein content and metabolisable energy (López et al. 2013). Tiller height is positively related with grazing probability (Hodgkinson et al. 2011), and this variable is associated with number of leaves per tiller and lamina length as was shown in an experiment of a mixed pasture of *L. perenne* and *B. valdivianus*, favouring *B. valdivianus* selection over *L. perenne* (López et al. 2013). The former study was evaluated with European wild boar and the second with cows, however sheep have a greater possibility to select from different parts of plants, because they hold forage with their lips and cut it with the teeth. Griffiths et al. (2003) reported a similar result where cows showed preference in relation to sward height but not quantity of leaf mass.

Mammals select what to eat through the smell and flavour of foods and they associate them with nutritive value of the food and eat for nutrients rather than taste (Forbes and Kyriazakis, 1995). Lambs when they have to choose between a concentrated and a lower density nutrient food, select a great proportion of high fibre food (i.e. forages) (Cooper and Kyriazakis, 1993). Their behaviour is based on the requirement of a healthy rumen to avoid disease (e.g. rumen acidity) due to high concentration of volatile fatty acid in the rumen (Cooper and Kyriazakis, 1993). Even though legumes offer more nutrients per kg of dry matter and intake rate can be maximised, when sheep grazed *L. perenne-T. repens* monocultures (free accessibility to both pastures) they showed a partial preference for clover and a preference for a mixed diet, as their diet had 20–30% of the grass component (Parsons et al., 1994).

2.9 SUMMARY

In New Zealand pastoral systems, drought tolerant strategy perennial grasses can alleviate the low herbage mass production during summer and constrained persistence. Grazing frequency of defoliation is a key factor to incorporate these species into the traditional binary *L. perenne* and *T. repens* sowing pastures. Leaf regrowth stage is a suitable approach to set defoliation events in grass-based pastures, as assures high production, persistence, and high nutritive quality. However, there is a gap in the literature on the grazing criteria to use in mixed pastures comprised of two perennial grass species that differ in morphophysiological growth. Mixing high competitor species with high stress tolerance attributes species could allow for high herbage growth under high resources availability but, also, under low resource conditions (e.g., high and low soil water availabilities).

Bromus valdivianus is drought resistant species and its production and quality, as a monoculture, are similar to *L. perenne*. It has the potential to explore a different niche than *L. perenne* (i.e., deep soil exploration) and complement it through an asynchrony herbage growth throughout the year. In order to reach high species persistence and production, the defoliation frequency should be close to their optimal leaf regrowth stage. A common window opportunity for defoliation of both species in the mixture, both species should be able to display their functional traits in the pastoral ecosystem, raising the threshold in production and quality, but above all, offering forage to animals throughout the year, avoiding the strong growth seasonality found in a *L. perenne* and *T. repens* pasture.

Animals are able to modify the interspecific competition between the species by grazing selectively. Although *B. valdivianus* had shown high nutritive values, little is known about animal preference for the species. Its morphological structure, tiller size and number, differs from *L. perenne* and could impair the grow advantages of the former during the dry season. There is much evidence about animal preference between contrasting species, such as grass, legumes and forbs; but a lesser understanding on the animal preference between these two temperate grasses, which growth patterns occur across seasons. The frequency of defoliation and the animal behaviour could modify species succession in a diverse pasture with positive or negative effects on pasture production and persistence.

2.10 REFERENCES

Allden, W., & McD. Whittaker, I. (1970). The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. *Australian Journal of Agricultural Research, 21*, 755-766.

Allen, V. G., Batello, C., Berretta, E. J., Hodgson, J., Kothmann, M., Li, X., ... & Sanderson, M. (2011). An international terminology for grazing lands and grazing animals. *Grass and forage science*, *66*(1), 2.

Badeck, F. W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J., & Sitch, S. (2004). Responses of spring phenology to climate change. *The New Phytologist, 162*, 295-309.

Balfourier, F., Imbert, C., & Charmet, G. (2000). Evidence for phylogeographic structure in Lolium species related to the spread of agriculture in Europe. A cpDNA study. T*heoretical and Applied Genetics, 101*, 131-138.

Ballaré, C.L., & Casal, J. J. (2000). Light signals perceived by crop and weed plants. *Field Crops Research, 67,* 149-160.

Balocchi, O. A., & López, I. F. (2009). Herbage production, nutritive value and grazing preference of diploid and tetraploid perennial ryegrass cultivars (*Lolium perenne* L.). *Chilean Journal of Agricultural Research, 69,* 331-339.

Barker, D. J., & Caradus, J. R. (2001). Adaptation of forage species to drought. In *Proceedings of the XIX International Grassland Congress—Grassland Ecosystems: An Outlook into the 21st Century (2001)* (Vol. 19, pp. 241-246).

Barker, G. M., Pottinger, R. P., & Addison, P. J. (1984). Effect of Argentine stem weevil on productivity of grasses in the Waikato. *New Zealand Journal of Agricultural Research, 27,* 93-101.

Bartholomew, P., & Williams, R. (2006). Effects of exposure to below-freezing temperatures, soil moisture content and nitrogen application on phyllochron in cool-season grasses. *Grass and Forage Science, 61,* 146-153.

Belsky, A. (1986). Does herbivory benefit plants? A review of the evidence. *The American Naturalist, 127,* 870-892.

Berone, G. D., Lattanzi, F. A., Colabelli, M. R., & Agnusdei, M. G. (2007). A comparative analysis of the temperature response of leaf elongation in *Bromus stamineus* and *Lolium perenne p*lants in the field: intrinsic and size-mediated effects. *Annals of Botany, 100,* 813-820.

Bluett, S. J., Thom, E. R., Clark, D. A., Macdonald, K. A., & Minneé, E. M. K. (2005). Effects of perennial ryegrass infected with either AR1 or wild endophyte on dairy production in the Waikato. *New Zealand Journal of Agricultural Research, 48,* 197-212.

Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research, 112,* 119-123.

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell Environ, 40,* 4-10.

Bonhomme, R. (2000). Bases and limits to using 'degree. day'units. *European journal of agronomy, 13,* 1-10.

Briske, D., Boutton, T., & Wang, Z. (1996). Contribution of flexible allocation priorities to herbivory tolerance in C 4 perennial grasses: an evaluation with 13 C labeling. *Oecologia, 105,* 151-159.

Briske, D., & Hendrickson, J. (1998). Does selective defoliation mediate competitive interactions in a semiarid savanna? A demographic evaluation. *Journal of Vegetation Science, 9,* 611-622.

Briske, D., & Stuth, J. (1982). Tiller defoliation in a moderate and heavy grazing regime. *Journal of Range Management, 35*(4), 511-514.

Brougham, R. (1956). Effect of intensity of defoliation on regrowth of pasture. *Australian journal of agricultural research, 7,* 377-387.

Calvache, I., Balocchi, O., Alonso, M., Keim, J.P., & López, I. (2020). Thermal Time as a Parameter to Determine Optimal Defoliation Frequency of Perennial Ryegrass

(*Lolium perenne* L.) and Pasture Brome (*Bromus valdivianus* Phil.). *Agronomy, 10*(5), 620.

Casal, J. J., Sanchez, R. A., & Deregibus, V. A. (1986). The effect of plant density on tillering: the involvement of R/FR ratio and the proportion of radiation intercepted per plant. *Environmental and Experimental Botany*, *26*(4), 365-371.

Cave, L. M., Kenyon, P. R., Morris, S. T., López-Villalobos, N., & Kemp, P. D. (2015). Ewe lamb diet selection on plantain (*Plantago lanceolata*) and on a herb and legume mix, including plantain, chicory (*Cichorium intybus*), red clover (Trifolium pratense) and white clover (*Trifolium repens*). *Animal Production Science, 55*(4), 515-525.

Chapman, D., & Lemaire, G. (1993). Morphogenetic and structural determinants of plant regrowth after defoliation. p. 94–104. In MJ Baker et al. (Eds.). *Proceedings of the XVII International Grassland Congress, Palmerston North and Rockhampton, 1993* (pp. 95-104). Sir Publishing.

Chapman, D., Muir, P., & Faville, M. (2015). Persistence of dry matter yield among New Zealand perennial ryegrass (*Lolium perenne* L.) cultivars: insights from a long-term data set. *Journal of New Zealand Grasslands 77,* 177-184.

Charles, A., & Valentine, J. (1978). A comparison of diploid and tetraploid *Lolium perenne* L. sown alone and in mixtures with particular reference to the effect of treading. T*he Journal of Agricultural Science, 91,* 487-495.

Charton, J., & Stewart, A. (1999). Pasture species and cultivars used in New Zealand – a list. *Proceedings of the New Zealand Grassland Association, 61*, 147-166.

Clark, D. (2011). Changes in pastoral farming practices and pasture persistence-a review. *NZGA: Research and Practice Series, 15,* 7-13.

Clayton, W. D., & Renvoize, S. A. (1986). Genera graminum. Grasses of the world. Genera graminum. *Grasses of the World, 13.*

Cooper, S., & Kyriazakis, I. (1993). The diet selection of lambs offered food choices of different nutrient density. *Proceedings of the British Society of Animal Production (1972), 1993,* 196-196.

de Faccio Carvalho, P. C. (2013). Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? *Tropical Grasslands-Forrajes Tropicales, 1,* 137-155.

De Wit, C. T. (1960). *On competition,* Pudoc.

Descalzi, C., Balocchi, O., López, I., Kemp, P., & Dörner, J. (2018). Different soil structure and water conditions affect the growing response of *Lolium perenne* L. and *Bromus valdivianus* Phil. growing alone or in mixture. *Journal of soil science and plant nutrition, 18,* 617-635.

Donaghy, D. J., & Fulkerson, W. J. (1998). Priority for allocation of water-soluble carbohydrate reserves during regrowth of Lolium perenne. *Grass and Forage Science, 53,* 211-218.

Donaghy, D. J., Turner, L. R., & Adamczewski, K. A. (2008). Effect of defoliation management on water-soluble carbohydrate energy reserves, dry matter yields, and herbage quality of tall fescue. *Agronomy Journal, 100*(1), 122-127.

Donald, C. M. (1963). Competition among crop and pasture plants. In *Advances in agronomy* (Vol. 15, pp. 1-118). Academic Press.

Durand, J.-L., Onillon, B., Schnyder, H., & Rademacher, I. (1995). Drought effects on cellular and spatial parameters of leaf growth in tall fescue. *Journal of experimental botany, 46,* 1147-1155.

Durand, J. L., Schäufele, R., & Gastal, F. (1999.) Grass leaf elongation rate as a function of developmental stage and temperature: morphological analysis and modelling. *Annals of Botany, 83,* 577-588.

Duru, M., Cruz, P., Ansquer, P., & Navas, M.L. (2014). Standing herbage mass: An integrated indicator of management practices for examining how fertility and defoliation regime shape the functional structure of species-rich grasslands. *Ecological Indicators, 36,* 152-159.

Edwards, G., Parsons, A., Penning, P., & Newman, J. (1995). Relationship between vegetation state and bite dimensions of sheep grazing contrasting plant species and its implications for intake rate and diet selection. *Grass and Forage Science, 50,* 378-388.

Elliott, J., Deryng, D., Muller, C., Frieler, K., Konzmann, M., Gerten, D., Glotter, M., Florke, M., Wada, Y., Best, N., Eisner, S., Fekete, B. M., Folberth, C., Foster, I., Gosling, S. N., Haddeland, I., Khabarov, N., Ludwig, F., Masaki, Y., Olin, S., Rosenzweig, C., Ruane, A. C., Satoh, Y., Schmid, E., Stacke, T., Tang, Q., & Wisser, D. (2014). Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proceedings of the National Academy of Sciences of the United States of America ,111,* 3239-44.

Emmans, G. C. (1991). Diet selection by animals: theory and experimental design. *Proceedings of the Nutrition Society, 50*(1), 59-64.

Ewel, J. J. (1991). Yes, we got some bananas. *Conservation Biology, 5,* 423-425.

Feldhake, C. M., & Belesky, D. (2009). Photosynthetically active radiation use efficiency of *Dactylis glomerata* and *Schedonorus phoenix* along a hardwood tree-induced light gradient. *Agroforestry systems, 75,* 189-196.

Finn, J. A., Suter, M., Haughey, E., Hofer, D., & Lüscher, A. (2018). Greater gains in annual yields from increased plant diversity than losses from experimental drought in two temperate grasslands. *Agriculture, Ecosystems & Environment, 258,* 149-153.

Fletcher, L. (1999), Non-toxic" endophytes in ryegrass and their effect on livestock health and production. Ryegrass endophyte: an essential New Zealand symbiosis. *Grassland Research and Practice Series, 7,* 133œ139.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J.S., Johnston, M., Mueller, N. D., O'Connel,l C., Ray, D. K., & West, P. C. (2011). Solutions for a cultivated planet. *Nature, 478*(7369), 337-342.

Forbes, J. M., & Kyriazakis, I. (1995). Food preferences in farm animals: why don't they always choose wisely? *Proceedings of the Nutrition Society, 54*(2), 429-440.

Fournier, C., Durand, J. L., Ljutovac, S., Schaufele, R., Gastal, F., & Andrieu, B. (2005). A functional-structural model of elongation of the grass leaf and its relationships with the phyllochron. N*ew Phytol, 166,* 881-94.

Frame, J. (1973). The yield response of a tall fescue/white clover sward to nitrogen rate and harvesting frequency. *Grass and Forage Science, 28,* 139-148.

Fuhlendorf, S. D., & Smeins, F. E. (1997). Long-term vegetation dynamics mediated by herbivores, weather and fire in a Juniperus-Quercus savanna. *Journal of Vegetation Science, 8,* 819-828.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture, 41,* 261-275.

García-Favre, J., Zanoniani, R., Cadenazzi, M., & Boggiano, P. (2017). Incidence of biological and edaphic variables on the establishment of forage mixtures. *Agro Sur, 45,* 3-10.

Gastal, F., & Lemaire, G. (2015). Defoliation, Shoot Plasticity, Sward Structure and Herbage Utilization in Pasture: Review of the Underlying Ecophysiological Processes. *Agriculture, 5,* 1146-1171.

Gatti, M. L., Ayala Torales, A. T., Cipriotti, P. A., & Golluscio, R. A. (2013). Leaf and tiller dynamics in two competing C 3 grass species: influence of neighbours and nitrogen on morphogenetic traits. *Grass and Forage Science, 68*(1), 151-164.

Gatti, M. L., Ayala Torales, A. T., Cipriotti, P. A., & Golluscio R. A. (2017). Effects of defoliation frequency and nitrogen fertilization on the production and potential for persistence of *Dactylis glomerata* sown in multispecies swards. *Grass and Forage Science, 72*, 489-501.

Gause, G. F. (2003). *The struggle for existence.* Courier Corporation.

Gibson, D. J., & Newman, J. A. (2019). Grasslands and climate change: An overview. In D.J. Gibson & J.A. Newman (Eds.), *Grasslands and climate change* (pp. 3–18). Cambridge University Press.

Graff, P., & Aguiar, M. R. (2017). Do species' strategies and type of stress predict net positive effects in an arid ecosystem? *Ecology, 98*, 794-806.

Griffiths, W. M., Hodgson, J., & Arnold, G. C. (2003). The influence of sward canopy structure on foraging decisions by grazing cattle. I. Patch selection. *Grass and Forage Science, 58*(2), 112-124.

Grime, J. P. (1977) .Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist, 111*, 1169-1194.

Grime, J. P. (2002). Declining plant diversity: empty niches or functional shifts?. *Journal of Vegetation Science, 13*(4), 457-460.

Harmer, M., Stewart, A., & Woodfield, D. (2016). Genetic gain in perennial ryegrass forage yield in Australia and New Zealand. *Journal of New Zealand Grasslands, 78,* 133-138.

Harris, W., Forde, B., & Hardacre, A. (1981). Temperature and cutting effects on the growth and competitive interaction of ryegrass and paspalum: 1. Dry matter production, tiller numbers, and light interception. *New Zealand journal of agricultural research ,24,* 299-307.

Hazard, L., & Ghesquière, M. (1995). Evidence from the use of isozyme markers of competition in swards between short-leaved and long-leaved perennial ryegrass. *Grass and Forage Science, 50,* 241-248.

Henessy, K., Fitzharris, B., Bates, N., Harvey, S., Howden, M., Hughes, L., Salinger J., & Warrick, R. (2007). Australia and New Zealand, climate change 2007: Impacts, adaptation and vulnerability. In M. Parry, O. Canzani, J. Palutikof, P. van der Linden, & C. Hanson (Eds.), *Contributions of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The quarterly review of biology, 67,* 283-335.

Hernández-Agramonte, I. M., Semmartin, M., Omacini, M., Durante, M., Gundel, P. E., & De Battista, J. (2018). A fungal endophyte of a palatable grass affects preference of large herbivores. *Austral Ecology, 43,* 172-179.

Hodgkinson, S. M., Cárcamo, A., & López, I. (2011). Selective grazing of Lolium perenne and Plantago lanceolata by growing European wild boar (Sus scrofa L.) in a semi-extensive system. *Livestock Science*, *140*(1-3), 268-274.

Hodgson, J. (1979). Nomenclature and definitions in grazing studies. *Grass and forage science*, *34*(1), 11-17.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., Lüscher, A., & Bennett, J. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology, 53*, 1023-1034.

Hooper, D. U., Chapin, F., Ewel, J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J., Lodge, D., Loreau, M., & Naeem, S. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological monographs*, *75*, 3-35.

Hooper, D. U., & Dukes, J. S. (2003). Overyielding among plant functional groups in a long-term experiment. *Ecology Letters ,7,* 95-105.

Hoveland, C. (1997). Problems in establishment and maintenance of mixed swards. In *International Grasslands Congress* (pp. 411-416).

Huber, H., Lukács, S., & Watson, M. A. (1999). Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecology, 141,* 107-115.

Husse, S., Huguenin-Elie, O., Buchmann, N., & Lüscher, A. (2016). Larger yields of mixtures than monocultures of cultivated grassland species match with asynchrony in shoot growth among species but not with increased light interception. *Field Crops Research, 194,* 1-11.

Insua, J. R., Agnusdei, M. G., Utsumi, S. A., & Berone, G. D. (2018). Morphological, environmental and management factors affecting nutritive value of tall fescue (*Lolium arundinaceum*). *Crop and Pasture Science, 69*(11), 1165-1172.

Kaufononga, S., Donaghy, D. J., Hendriks, S. J., Matthew, C., Kemp, P. D., & Cranston, L. M. (2017). Comparative response of tall fescue and perennial ryegrass swards to variation in defoliation interval and height. *New Zealand Journal of Agricultural Research, 60,* 363-375.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science, 70,* 283-295.

Kemp, D., & King, W. (2001). Plant competition in pastures–implications for management. *Competition and succession in pastures,* 85-102.

Kemp, D. R., & Culvenor, R. A. (2010). Improving the grazing and drought tolerance of temperate perennial grasses. *New Zealand Journal of Agricultural Research, 37,* 365-378.

Kenny, G. J., Warrick, R. A., Campbell, B. D., Sims, G. C., Camilleri, M., Jamieson, P. D., Mitchell, N. D., McPherson, H. G., & Salinger, M. J. (2000). Investigating climate change impacts and thresholds: An application of the CLIMPACTS Integrated Assessment Model for New Zealand agriculture. *Climatic Change, 46*(1), 91-113.

Kiaer, L. P., Weisbach, A. N., Weiner, J., Gibson, D. (2013). Root and shoot competition: a meta-analysis. *Journal of Ecology, 101*, 1298-1312.

Korner, C. (1991). Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. *Functional ecology*, 162-173.

Kyriazakis, I., & Oldham, J. D. (1993). Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen× 6.25) requirements. *British journal of Nutrition*, *69*(3), 617-629.

Larcher, W. (2003). *Physiological plant ecology: ecophysiology and stress physiology of functional groups.* Springer Science & Business Media.

Launchbaugh, K. (1996). Biochemical aspects of grazing behavior. In: J. Hodgson & A. W. Illius (Eds.), *The ecology and management of grazing systems* (pp. 159-184). CABI.

Leafe, E. (1988). Introduction—the history of improved grasslands. In Jones, M.C., & Lazenby, A. (Eds), *The Grass Crop* (pp. 1-23). Springer.

Lee, J., Thom, E., Waugh, C., Bell, N., McNeill, M., Wilson, D., & Chapman D. (2017). Trajectory and causes of decline in the botanical composition of dairy-grazed pasture in the Waikato. *Journal of New Zealand Grasslands,* 89-96.

Lee ,J. M., Matthew, C., Thom, E. R., & Chapman, D. F. (2012). Perennial ryegrass breeding in New Zealand: A dairy industry perspective. *Crop and Pasture Science, 63*(2), 107-127.

Lemaire, G. (2001). Ecophysiology of grasslands: dynamic aspects of forage plant populations in grazed swards. *International Grassland Congress, 19,* 29-37.

Litherland, A., Woodward, S., Stevens, D., McDougal, D., Boom, C., Knight, T., & Lambert M. (2002). Seasonal variations in pasture quality on New Zealand sheep and beef farms. In *Proceedings-New Zealand Society of Animal Production* (Vol. 62, pp. 138-142). New Zealand Society of Animal Production; 1999.

López, I., Balocchi, O., Lailhacar, P., & Oyarzún, C. (1997). Caracterización de sitios de crecimiento de seis especies pratenses nativas y naturalizadas del Dominio Húmedo de Chile. *Agro sur, 25,* 62-80.

López, I. F., Balocchi, O. A., Alvarez, X., Flores, P., & Latrille, L. (2016). Selectividad de Bromus valdivianus Phil., Lolium perenne L. y Agrostis capillaris L. por vacas lecheras en pastoreo. *Agro Sur, 44,* 53-65.

López, I. F., Kemp, P. D., Dörner, J., Descalzi, C. A., Balocchi, O. A., & García, S. (2013). Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. and *Lolium perenne* L. plants under water restriction. *Journal of Agronomy and Crop Science, 199,* 449-459.

Loreau, M. (1998). Separating sampling and other effects in biodiversity experiments. *Oikos,* 600-602.

Marquard, E., Weigelt, A., Temperton, V. M., Roscher, C., Schumacher, J., Buchmann, N., Fische,r M., Weisser, W.W., & Schmid, B. (2009). Plant species richness and functional composition drive overyielding in a six-year grassland experiment. E*cology, 90,* 3290-3302.

Maschinski, J., & Whitham, T. G. (1989). The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist, 134,* 1-19.

Matthew, C., Agnusdei, M. G., Assuero, S. G., Sbrissia, A. F., Scheneiter, O., & Da Silva, S. C. (2013) State of knowledge in tiller dynamics. In *Proceedings of the XXII*

International Grassland Congress, Sydney, Australia, 15–19 September 2013 (pp. 1041–1044).

Matthew, C., Lemaire, G., Sackville Hamilton, N. R., & Hernandez-Garay, A. (1995). A modified self-thinnng to describe size/density relationship for defoliated swards. *Annals of Botany, 76*, 579-587.

McDonagh, J., O'Donovan, M., McEvoy, M., & Gilliland, T. (2016) Genetic gain in perennial ryegrass (Lolium perenne) varieties 1973 to 2013. *Euphytica*, *212,* 187-199.

McGilchrist, C. A., & Trenbath, B. R. (1971). A revised analysis of plant competition experiments. *Biometrics*, 659-671.

McMaster, G. S., & Wilhelm, W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and forest meteorology, 87*, 291-300.

McNaughton, S. (1979). Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *The American Naturalist, 113,* 691-703.

McNaughton, S., Banyikwa, F. F., & McNaughton, M. (1998). Root biomass and productivity in a grazing ecosystem: the Serengeti. *Ecology*, *79*,587-592.

McNaughton, S., & Wolf, L. (1970). Dominance and the niche in ecological systems. *Science, 167*, 131-139.

McNaughton, S. (1977). Diversity and stability of ecological communities: a comment on the role of empiricism in ecology. *The American Naturalist, 111*,515-525.

McNaughton, S. J., Oesterheld, M., Frank, D. A., & Williams, K. (1989). Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature, 341*(6238), 142-144

Nabinger, C. (1998). Princípios de manejo e produtividade de pastagens. *Ciclo de palestras em produção e manejo de bovinos de corte, 3*, 54-107.

Nelson, C. (2000) Shoot morphological plasticity of grasses: leaf growth vs. tillering. In G. Lemaire, J. Hodgson, A. Moraes, P.C. de Cavalho, & C. Nabinger (Eds.), *Grassland ecophysiology and grazing ecology* (pp. 101-126). CABI Publishing.

Nie, Z., Chapman, D., Tharmaraj, J., & Clements, R. (2004). Effects of pasture species mixture, management, and environment on the productivity and persistence of dairy pastures in south-west Victoria. *Australian Journal of Agricultural Research, 55*, 637-643.

Noy-Meir, I. (1975). Stability of grazing systems: an application of predator-prey graphs. *The Journal of Ecology,* 459-481.

Nurjaya, I., & Tow, P. (2001) Genotype and environmental adaptation as regulators of competitiveness. In P.G Tow & A. Lazenby (Eds.), *Competition and succession in pastures*. CAB Int. Press.

O'connor, T. (1991) Local extinction in perennial grasslands: a life-history approach. *The American Naturalist*, *137,* 753-773.

Ordoñez, I., López, I., Kemp, P., Donaghy, D., Herrmann, P., Hernández, F., & Bhatia, S. (2017) .Pasture brome (*Bromus valdivianus*) leaf growth physiology: a six-leaf grass species. *Agron. NZ, 47,* 13-22.

Ordóñez, I., López, I.F., Kemp, P. D., Descalzi, C. A., Horn, R., Zúñiga, F., Dec, D., & Dörner, J. (2018). Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. *Soil and Tillage Research 178,* 55-64.

Overpeck, J. T. (2013). The challenge of hot drought. *Nature, 503,* 350-351.

Parry, M., Canziani, O., Palutikof, J., van der Linden, P. J., & Hanson, C. E. (2007). C*limate change 2007: impacts, adaptation and vulnerability*. Cambridge University Press Cambridge.

Parsons, A. (1988). The effects of season and management on the growth of grass swards.In M.B. Jones & A. Lazenby (Eds), *The grass crop* (pp. 129-177). Springer.

Parsons, A., Leafe, E., Collett, B., Penning, P., & Lewis, J. (1983). The physiology of grass production under grazing. II. Photosynthesis, crop growth and animal intake of continuously-grazed swards. *Journal of applied ecology,* 127-139.

Parsons, A., Newman, J., Penning, P., Harvey, A., & Orr, R. (1994). Diet preference of sheep: effects of recent diet, physiological state and species abundance. *Journal of animal ecology,* 465-478.

Parsons, A., & Penning, P. (1988). The effect of the duration of regrowth on photosynthesis, leaf death and the average rate of growth in a rotationally grazed sward. *Grass and forage science, 43,* 15-27.

Peltzer, D. A., & Wilson, S. D. (2000). Competition and Environmental Stress in Temperate Grasslands. In P. G. Tow & A. Lazenby (Eds.), *Competition and succession in pastures* (pp. 193-212). CAB International 2001.

Penning, P., Parsons, A., Orr, R., & Treacher, T. (1991). Intake and behaviour responses by sheep to changes in sward characteristics under continuous stocking. *Grass and Forage Science, 46,* 15-28.

Roscher, C., Kutsch, W., & Schulze, E. D. (2011). Light and nitrogen competition limit Lolium perenne in experimental grasslands of increasing plant diversity. *Plant Biology, 13,* 134-144.

Rosenthal, J., & Kotanen, P. (1994). Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution, 9,* 145-148.

Rutter, S. M. (2006). Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. *Applied Animal Behaviour Science, 97,* 17-35.

Sackville-Hamilton, N. (2001). Measurement of competition and competition effects in pastures. In P.G. Tow & A. Lazenby (Eds), *Competition and succession in pastures* (pp:15-42). CAB International 2001.

Sanderson, M. A., Skinner, R.H., BArker, D.J., Edwards, G.R., Tracy, B.F., & Wedia, D.A. (2004). Plant Species Diversity and Management of Temperate Forage and Grazing Land Ecosystem. *Crop Scienc,e 44*(4), 1132-1144.

Seager, N. G., Kemp, P. D., & Chu, A. C. (1992). Effect of root and shoot competition from established hill-country pasture on perennial ryegrass. *New Zealand journal of agricultural research, 35,* 359-363.

Sekiya, N., Araki, H., & Yano, K. (2010). Applying hydraulic lift in an agroecosystem: forage plants with shoots removed supply water to neighboring vegetable crops. *Plant and Soil, 341,* 39-50.

Serraj, R., & Sinclair, T. (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant, cell & environment, 25,* 333-341.

Silvertown, J., Lines, C. E., & Dale, M. P. (1994). Spatial competition between grasses--Rates of mutual invasion between four species and the interaction with grazing. *Journal of Ecology,* 31-38.

Skinner, R., & Nelson, C. (1995). Elongation of the grass leaf and its relationship to the phyllochron. *Crop Science, 35,* 4-10.

Smetham, M. (1990). Pasture management. In R.H.M. Langer (Ed.), *Pastures—Their Ecology and Management* (pp. 197–240). Oxford University Press.

Stewart, A. (1996). Potential value of some Bromus species of the section Ceratochloa. *New Zealand Journal of Agricultural Research*, 39, 611-618.

Stuth, J. W. 1991. Foraging behavior. In R.K. Heitschmidt and J.W. Stuth (Eds.). *Grazing management: an ecological approach* (pp. 65-83). Tiiber Fvess, Portland, ore.

Tejera, M., Speranza, P., Astigarraga, L., & Picasso V. (2016). Forage biomass, soil cover, stability and competition in perennial grass-legume pastures with differentPaspalumspecies. *Grass and Forage Science, 71*, 575-583.

Teughels, H., Nijs, I., Van Hecke, P., Impens, I. (1995). Competition in a global change environment: the importance of different plant traits for competitive success. *Journal of Biogeography*, 297-305.

Tilman, D. (2001) Functional diversity. *Encyclopedia of biodiversity, 3*, 109-120.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science, 277*, 1300-1302.

Tor-Agbidye, J., Blythe, L., & Craig, A. (2001). Correlation of endophyte toxins (ergovaline and lolitrem B) with clinical disease: fescue foot and perennial ryegrass staggers. *Veterinary and Human Toxicology, 43*, 140-146.

Tow, P. G., & Lazenby, A. (2000). *Competition and succession in pastures*. CABI.

Tozer, K. N., Carswell, K., Griffiths, W. M., Crush, J. R., Cameron, C. A., Chapman, D. F., Popay, A., & King, W. (2017). Growth responses of diploid and tetraploid perennial ryegrass (*Lolium perenne*) to soil-moisture deficit, defoliation and a root-feeding invertebrate. *Crop and Pasture Science, 68*(7), 632-642.

Turner, L., Donaghy, D., Lane, P., & Rawnsley, R. (2006a). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 2. Nutritive value. *Grass and forage science, 61*, 175-181.

Turner, L. R., Donaghy, D. J., Lane, P. A., & Rawnsley, R. P. (2006b). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 1. Regrowth, tillering and water-soluble carbohydrate concentration. *Grass and Forage Science 61*, 164-174.

Turner, N. (1986). Adaptation to Water Deficits: a Changing Perspective. *Functional Plant Biology 13*, 175-190.

Vicari, M., & Bazely, D.R. (1993). Do grasses fight back? The case for antiherbivore defences. Trends in ecology & evolution 8:137-141.

Vitousek, P., & Hooper, D. (1994). Biological diversity and terrestrial ecosystem biogeochemistry, In *Biodiversity and ecosystem function* (pp. 3-14). Springer.

Vojtech, E., Loreau, M., Yachi, S., Spehn, E. M., & Hector, A. (2008). Light partitioning in experimental grass communities. *Oikos, 117*, 1351-1361.

Volaire, F. (2018). A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Global Change Biology, 24*, 2929-2938.

Volaire, F., Barkaoui, K., & Norton, M. (2014). Designing resilient and sustainable grasslands for a drier future: Adaptive strategies, functional traits and biotic interactions. E*uropean Journal of Agronomy, 52,* 81-89.

Westoby, M., Walker, B., & Noy-Meir, I. (1989). Opportunistic management for rangelands not at equilibrium. *Rangeland Ecology & Management/Journal of Range Management Archives, 42*(4), 266-274.

Wilhelm, W., & McMaster, G. S. (1995). Importance of the phyllochron in studying development and growth in grasses. *Crop Science, 35,* 1-3.

Wilkins, P., & Humphreys, M. (2003). Progress in breeding perennial forage grasses for temperate agriculture. *The Journal of Agricultural Science, 140*, 129-150.

Wilson, J. B. (1988). Shoot competition and root competition. *Journal of applied ecology,* 279-296.

Wright, S. D., & Mcconnaughay, K. D. (2002). Interpreting phenotypic plasticity: the importance of ontogeny. *Plant Species Biology, 17,* 119-131.

CHAPTER 3

The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels

---

Pasture defoliation frequency based on leaf regrowth stage, according to the number of fully developed leaves, enhances pasture growth and persistence. Therefore, the energy replenishment in the tiller base is an important indicator of the minimum number of leaves per tiller on perennial grasses required to be defoliated. *Bromus valdivianus* optimal leaf regrowth stage and its effect on growth response under low soil water availabilities need to be assessed. The objectives of the present chapter were to determine *Bromus valdivianus* herbage mass and root production, and energy reserve levels (i.e., water soluble carbohydrates) under three contrasting defoliation frequencies and two soil-water restriction levels in a glasshouse study.

Agronomy, Published. Reference: García-Favre, J., López, I. F., Cranston, L. M., Donaghy, D. J., & Kemp, P. D. (2021). The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels. *Agronomy*, *11*(2), 300.

3.1 ABSTRACT

Pasture brome (*Bromus valdivianus* Phil.) has the potential to increase current levels of herbage production and pasture persistence in New Zealand dryland (without irrigation), well-drained soils. However, there is little literature on the effect of defoliation management on growth of this grass under contrasting soil-water restriction levels. The growth physiology and performance of pasture brome were evaluated in pots in a glasshouse. Defoliation frequency (DF) treatments were applied based on three different accumulated growing degree-days (AGDD): 250, 500 and 1000 AGDD (high, medium, and low DF). At end of the first growing cycle (1000 AGDD), water availability was restricted to 20–25% of field capacity (FC) in half of the pots, while the other pots were maintained between 80–85% FC. Total accumulated herbage mass was positively related with the low DF and well-watered conditions ($p < 0.05$). At the final harvest, plants subjected to low DF had greater root mass than high and medium DF ($p < 0.05$). At each harvest, the leaf regrowth stage (LS) for low DF was 3.5, while for high and medium DF, the LS was 1.5 and 2.0; respectively. Tiller water-soluble carbohydrates were highest at the low DF and under 20–25% FC. Regardless of soil-water conditions, defoliation at 3.5 LS supports production, enhancing survival during a drought.

3.2 INTRODUCTION

Bromes (Bromus spp.) is a genus of grass species that include some useful forage types, as well as some weedy species. *Bromus valdivianus* Phil. (Bv) is native to the temperate humid region of Chile. It has similar agricultural attributes to perennial ryegrass *Lolium perenne* L. (accumulated herbage mass production, herbage quality) but produces more herbage mass during soil water restriction periods, such as summer (Keim et al., 2015). However, previous research on Bv in New Zealand has shown it produced less total herbage mass than other perennial grasses, such as *Dactylis glomerata* L. and *Festuca arundinacea* Schreb., when grown in hill country under dry summer conditions (Fasi et al., 2008). Further, it exhibited poorer survival than *D. glomerata* when sown in a mix with *Medicago sativa* L. in hill country in the Canterbury region with an average rainfall of 643 mm/year (Moot et al., 2019). However, in both of these studies, all the species were defoliated at the same time without considering species-specific defoliation intervals. The frequency and intensity of defoliation are key factors that determine the production and persistence of forage species (Harris, 1978). Unsuitable defoliation frequency leads to depletion of perennial grass species soluble carbohydrate reserves and low root mass resulting in poor survival during drought and slow recovery after it (Donaghy et al., 2008).

Defoliation frequency based on leaf regrowth stage (LS) aligns perennial grass species plant physiology with production and persistence, such that the regrowth post defoliation is based on the plant recovery of its water-soluble carbohydrate (WSC) reserves. This defoliation strategy supports persistence in fast-growing grass species (Fulkerson and Donaghy, 2001). The recovery of the WSC reserves in plants during the regrowth period after defoliation is what determines the lower range of LS recommended for grazing (Donaghy and Fulkerson, 1998). *Bromus valdivianus* Phil is a six-leaf species with an optimum LS range for defoliation between 3 and 4 (Ordoñez et al., 2017). However, that research did not address below-ground morphological changes, such as root biomass, or plant energy reserves (WSC in the stubble) in relation to LS defoliation criteria. It is well known that resource allocation to roots is not a priority immediately after defoliation for perennial grass species (Donaghy and Fulkerson, 1998; Turner et al., 2007). Further, frequent defoliation mostly affects elongation and survival of roots, resulting in a plant with a poor root system (Donaghy and Fulkerson, 1998), which is expected to negatively impact plant survival under water stress conditions.

Forage species have diverse strategies when faced with soil-water restriction and increasing temperatures. The primary strategies of plants are drought escape, dehydration avoidance and dehydration tolerance (Turner, 1986). Temperate grasses, subjected to moderate to low soil moisture levels, express dehydration avoidance through developing a deeper root and leaf osmotic adjustment. If soil moisture levels continue decreasing, the plants start a drought-survival phase, where growth is suppressed as the energy is allocated to the growing point of stems (dehydration tolerance) (Norton et al., 2016). A common strategy observed in some grasses, such as *Dactylis glomerata* L., to survive an intense drought is the accumulation of WSC in the meristem tissues of leaves (Volaire et al., 2020; Volaire et al., 2005). The reserve of WSC in the stubble also play a specific role in the fast recovery of growth when a drought ends (Thomas and James, 1999). Little is known about the interaction between defoliation frequency and soil water content on Bv growth and survival. It is hypothesised that infrequent defoliations would result in plants with more extensive root systems and a full replenishment of WSC that would allow them to survive low soil water availability. The aim of the present study was to evaluate Bv production, resource allocation within the plant and WSC reserve levels under three contrasting defoliation frequencies and two soil-water restriction levels in a glasshouse study.

3.3 MATERIALS AND METHODS

3.3.1 Treatments and Experimental Design

The experiment was in a glasshouse at Massey University's Plant Growth Unit, School of Agriculture and Environment (−40.37° latitude south and 175.61° longitude west) under natural light conditions from 9 October 2018 to 12 March 2019. A total of 66 pots of 8 L capacity (24 cm upper diameter, 17 cm lower diameter and 24 cm height) were filled with a mixture of 30% Manawatu silt loam soil and 70% fine sand to achieve sandy loam texture. The results of the chemical analysis of the substrate were as follows: pH 6.3 (1:2 [soil:water]), 35 mg L^{-1} Olsen P, 0.34 me 100 g^{-1} potassium, 2.4 me 100 g^{-1} calcium, 0.6 me $100g^{-1}$ magnesium, 3 me $100g^{-1}$ cation exchange capacity and 83 mg kg^{-1} sulphate sulphur. Based on this analysis, the following fertiliser was added to each 60 L of substrate: 120 g of slow-release formula (14% nitrogen (N), 5% P, 10% soluble potash, 0.5% magnesium, 3.2% sulphur, 1.6% iron and 0.3% manganese), 60 g short term formula (14% total N, 6% P, 11.6% potassium, 1% magnesium, 4% sulphur, 1% iron and 0.5% manganese), and 90 g of dolomite. In each pot, two seeds of Bv cv. Bareno were sown in 24 equidistant positions, with 40 mm between them. At the early plantlet establishment stage, the number of plants per pot was thinned to one plant per position.

A combination of three defoliation frequencies and two soil-water restriction levels (2 x 3 factorial arrangement; six treatments) were applied in a complete randomised block design with four replicates. A previous glasshouse study found that Bv needs less than 1000 accumulated growing degree-days (AGDD) to produce the six live leaves that each tiller maintains (Ordoñez et al., 2017). Based on that information, three defoliation frequency targets were applied: high (250 AGDD), medium (500 AGDD) and low (1000

AGDD). The temperature inside the glasshouse was recorded daily at 10 min intervals. Thus, AGDD was calculated adding the average of maximum and minimum temperatures per day, following the method by (McMaster & Wilhelm, 1997). The average temperature was 22.5 °C for the experimental period, while maximum and minimum were 27.8 °C and 17.2 °C; respectively.

Two water restriction levels were applied: 80–85% of field capacity (FC) and 20–25% FC. The 80–85% FC was the control for the soil water restriction treatment. The soil water contents were continuously monitored by ECH2O EC-5 soil-moisture sensors at 10 cm depth in the soil at the centre of 12 pots, recording data every 15 min. The soil-moisture levels in each treatment were readjusted daily by irrigation according to the following formula:

$$I = ([IC - WC]/100) \times BD \times SD \times PA$$

where I = irrigation (L m^{-2}); IC = irrigation criteria (vol. %); WC = substrate water content (vol. %); BD = bulk density (mg m^{-3}); SD = substrate depth (m); PA = pot area (0.045 m^2).

The period of water restriction was comparable to the total number of days per year with a soil-moisture deficit in Palmerston North based on data collected from 2001 to 2018 (67 days per year; collected from the NIWA/Ag Research Weather Station, Agent No. 21963, Palmerston North, 2019).

Following sowing, three experimental stages were implemented.

(a) From sowing to the first harvest (H0): During this period, the plants grew for 9 weeks without environmental restrictions. Each Bv tiller maintained 6 live leaves at the same time: 3 fully expanded leaves, 2 expanding leaves and 1 emerging leaf; when the 7th leaf appeared, the older leaf began to senesce. Thus, H0 occurred when the 6th Bv leaf appeared in the older tillers. At this moment, all the plants were defoliated to 50-mm height, marking the beginning of the experimental period.

(b) Period from H0 to H1: In this period, three defoliation frequency treatments were imposed. This period ended (H1) with all plants being manually defoliated and occurred when 1000 AGGD was achieved, meaning that the medium defoliation frequency was defoliated twice, and the high defoliation frequency was defoliated 4 times. This period was utilised as a pre-treatment cycle to adapt the plants to the different defoliation frequencies before the application of soil-water treatments.

(c) Period from H1 to H2: The soil-water restriction treatments were applied until the longest defoliation treatment had grown through one full regrowth cycle (1000 AGDD). During this period, the medium defoliation frequency was again defoliated twice, and the high defoliation frequency was again defoliated 4 times.

3.3.2 Evaluated Variables

The herbage and root measurements performed in the period H1–H2 were as follows: At H1, individual tillers were marked at their base with a paper clip in two plants located in the centre of each pot and every three days the leaf length, number of leaves and leaf appearance were recorded. The leaf length of an elongating leaf included the distance from the ligule of the previous fully expanded leaf to the lamina tip, and for a fully expanded leaf considered the distance from the ligule to its tip. This was performed in every leaf per marked tiller. If a marked tiller died, it was replaced with another tiller that visually represented the mean population. The number of tillers per plant was counted once a week in the two central plants of each pot. Herbage at each defoliation event was dried at 70 °C for 48 h or until constant weight was reached. At the end of the study, total herbage mass accumulation was calculated for each defoliation treatment by adding the dried herbage from each defoliation event (four events for high, two for medium, and one for low defoliation frequencies). At the end of the H2 period, the two marked tillers per pot were cut at ground level and the leaf area (considering only the lamina), leaf weight, and specific leaf area (SLA; leaf area/unit weight) were determined. Leaf area was measured using a leaf area meter (LI-COR 3100, area meter). In order to compare between defoliation treatments, the variables were standardised using growing degree days according to Tsimba et al. (2013): leaf area expansion (mm^2 $°Cd^{-1}$), SLA (mm^2 mg^{-1} $°Cd^{-1}$) and changes in leaf weight (mg $°Cd^{-1}$). These variables were calculated by dividing the H2 collected data by the thermal time elapsed in each defoliation treatment. Leaf elongation rate (mm $°Cd^{-1}$) was calculated as the sum of leaf length between H1 and H2 (measured every three days) divided by the thermal time in each defoliation treatment. The remaining sward in each pot was cut to 50 mm. The stubble (tiller base below the 50-mm defoliation height) was frozen with liquid nitrogen before freeze-drying. The freeze-dried samples were then weighed, ground through a 1-mm sieve and sent to the Massey University Nutrition Laboratory (Palmerston North, New Zealand) where WSC concentration was obtained using the colorimetric assays developed by Somogyi-Nelson (Nelson, 1944).

Above-ground foliage mass and below ground root mass per plant on a dry-weight basis were measured from one centre plant from each of three spare pots per treatment at H2. These spare pots, 18 in total, were kept under the same conditions and treatments as the experimental pots. The roots were washed and scanned at 400 dpi with a scanner (Perfection V800 Photo, Epson), and the images were analysed with Winrhizo software (ver. 2012b, Regent Instruments Inc., Quebec, Canada) to determine total root length, root surface area, root diameter and root volume.

3.3.3 Statistical Analysis

Using R Statistic (Team, 2013), a one-way analysis of variance (ANOVA) and least significant difference (LSD) were used to analyse statistical differences with a level of significance of 5% ($p = 0.05$). Principal component analysis (PCA) was performed to recognise differences among treatments, as well as to detect the extent that each variable explained the variation between treatments. First, all the variables were standardised and then the analysis conducted with prcomp function in R software (Team, 2013).

3.4 RESULTS

3.4.1 Herbage Mass and Tiller Components

Accumulated herbage mass per pot increased by 32% under low defoliation frequency, with no significant differences between the high and medium defoliation frequencies. Low soil-water levels reduced accumulated herbage mass by 47% (Table 3.1). Tiller number per plant was not affected by any of the treatments.

On an individual tiller basis, leaf area and SLA significantly increased under more frequent defoliation. As expected, leaf regrowth stage (LS) significantly increased (there were more leaves) as defoliation frequency decreased. There was no significant effect of defoliation frequency on leaf weight and leaf length.

Water restriction significantly decreased leaf area (30%), LS (25%), SLA (25%) and leaf length (25%) but had no significant effect on individual leaf weight. In addition, there was a significant interaction between defoliation frequency and water restriction, only on LS, with more leaves/tiller present for the combination of more water, and longer defoliation intervals (Table 3.1).

Table 3.1. Herbage mass accumulation per pot and tiller components of *Bromus valdivianus* Phil. grown under three defoliation frequencies (Df) (250 [high], 500 [medium] and 1000 [low] accumulated growing degree days) and two levels of soil water content (Wl) (80–85% and 20–25% of field capacity [FC]).

	Herbage mass (g DM pot^{-1})	Leaf area rate/tiller (mm^2 °Cd^{-1})	Leaf weight rate/tiller (mg °Cd^{-1})	Leaf regrowth stage	Specific leaf area rate (mm^2 mg^{-1} °Cd^{-1})	Leaf length rate (mm °Cd^{-1})	Tillers No./plant
Defoliation frequency (Df)							
250 (High)	2.3 b	2.24 a	0.17	1.34 c	0.054 a	0.31	4.69
500 (Medium)	2.7 b	1.89 ab	0.15	1.73 b	0.025 b	0.34	4.38
1000 (Low)	4.2 a	1.44 b	0.11	2.95 a	0.011 c	0.38	4.06
Significance	**	*	ns	***	***	ns	ns
Water level (Wl)							
20–25% FC	2.3 b	1.54 b	0.14	1.76 b	0.026 b	0.22 b	4.08
80–85% FC	3.9 a	2.18 a	0.15	2.29 a	0.034 a	0.46 a	4.67
Significance	**	**	ns	***	*	***	ns
Df×Wl							
250×20–25% FC	1.5	1.75	0.15	1.21 d	0.048	0.18	4.25
250×80–85% FC	3.0	2.73	0.18	1.44 d	0.060	0.44	5.13
500×20–25% FC	2.2	1.73	0.15	1.50 d	0.022	0.23	3.75
500×80–85% FC	3.2	2.04	0.15	1.97 c	0.028	0.45	5.00
1000×20–25% FC	3.1	1.12	0.12	2.44 b	0.009	0.27	4.25
1000×80–85% FC	5.2	1.77	0.14	3.47 a	0.013	0.49	3.88
Significance	ns	ns	ns	*	ns	ns	ns

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

3.4.2 Plant Structure at final Harvest (H2)

Most above-and below-ground traits, such as above-ground foliage mass, root length, root surface area and root volume were not significantly affected by defoliating the plants at either medium or low defoliation frequencies; however, all of these measurements were significantly reduced by defoliating at high frequency (Table 3.2). Root mass per plant was 1.5 times greater under low defoliation frequency than the medium, and 3 times greater than the high defoliation frequency. Root diameter was not significantly affected by defoliation frequency and water level, but all other traits were reduced under 20–25 % FC (Table 3.2). The interaction between main effects was not significant ($p > 0.05$) for above ground biomass, root mass, total root length, root surface area, root diameter, root volume and WSC.

Table 3.2. Above- and below-ground components of *Bromus valdivianus* Phil. subjected to three defoliation frequencies (250 [high], 500 [medium] and 1000 [low] accumulated growing degree days) and two soil-water level conditions: 80–85% field capacity (FC) and 20–25% FC, at final harvest (H2).

	Above Ground Foliage Mass (g DM plant^{-1})	Root Mass (g DM plant^{-1})	Total Root Length (cm)	Root Surface Area (cm^2)	Root Diameter (mm)	Root Volume (cm^3)	WSC (mg tiller^{-1})
Defoliation frequency							
250 (High)	0.49 b	0.18 c	1422.8 b	90.4 b	0.21	0.47 b	2.04 c
500 (Medium)	1.24 a	0.37 b	3061.7 a	225.2 a	0.23	1.33 a	3.22 b
1000 (Low)	1.45 a	0.55 a	4092.9 a	298.5 a	0.23	1.76 a	12.53 a
Significance	*	***	**	***	ns	***	***
Water level							
20–25% FC	0.80 b	0.31 b	2280.4 b	156.1 b	0.22	0.86 b	6.20 a
80–85% FC	1.32 a	0.42 a	3437.9 a	253.4 a	0.23	1.51 a	4.95 b
Significance	*	*	*	**	ns	**	***

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

There was a strong linear relationship between tiller base weight and total WSC (mg/tiller). The WSC significantly increased when the defoliation frequency decreased and was also greater by a factor of ~20% per tiller in plants growing at 20–25% FC, compared to those growing at 80–85% FC (Table 3.2). The highest value for WSC occurred under water restriction and low defoliation frequency (13.5 mg); while the lowest value occurred in plants defoliated at the high frequency under well-watered conditions (1.1 mg). In addition, tiller weight showed the same trend as WSC, increasing with a decrease in defoliation frequency, and with water restriction. However, plants defoliated at the low frequency did not exhibit WSC differences between both water treatments (Figure 3.1).

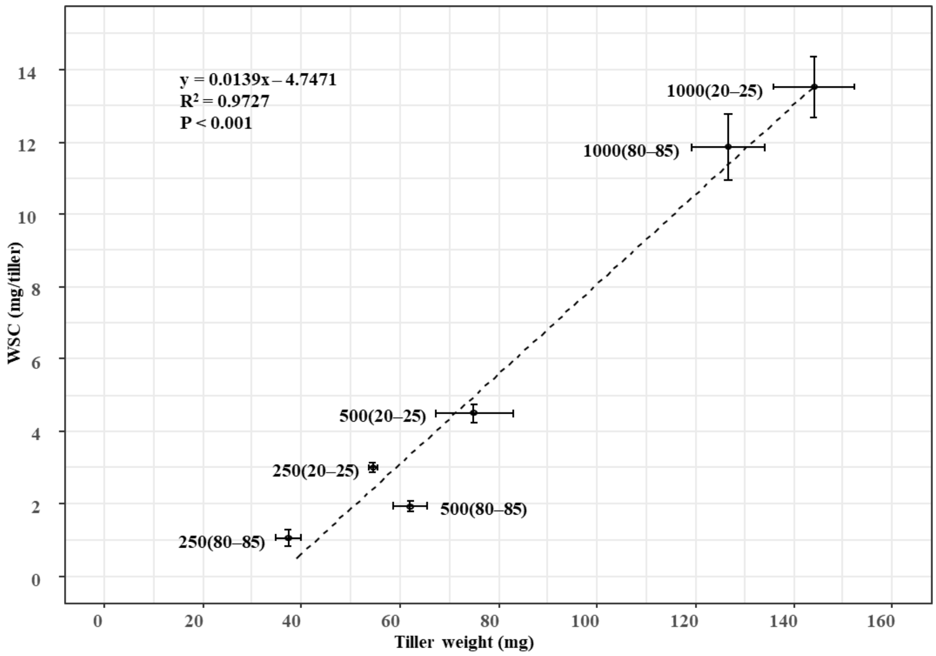


Figure 3.1. Relationship between weight of tiller base and water-soluble carbohydrates (WSC) per tiller (250 [high], 500 [medium] and 1000 [low] accumulated growing degree days) and two soil water levels: 80–85% field capacity (FC) and 20–25% FC. The standard error for WSC is shown as vertical bars, and the standard error for tiller weight is shown as horizontal bars.

In the PCA, the principal components 1 (PC1, which discriminated the treatments according to frequency of defoliation) and 2 (PC2, which discriminated the treatments according to soil water restriction), explained 90% of the total variation between treatments. Measured variables with coefficients higher than 0.25 were considered as a contributor to data pattern description. In PC1, which explained 72.3% of the variation, the variables that explained most of the differences between treatments were associated with root size and weight, LS, accumulated herbage mass and WSC. Plants defoliated at the medium defoliation frequency under well-watered conditions and plants defoliated at low defoliation frequency under both water treatments were positively associated with those variables. Then, plants growing under low defoliation frequency and 20–25% FC had similar root characteristics, LS and accumulated herbage mass to plants defoliated at the medium frequency under 80–85% FC. In PC2, which explained 18.3% of the variation, only WSC was positively related to 20–25% FC, while leaf area and leaf length rate were to 80–85% FC and to defoliations at the high and medium frequencies (Figure 3.2).

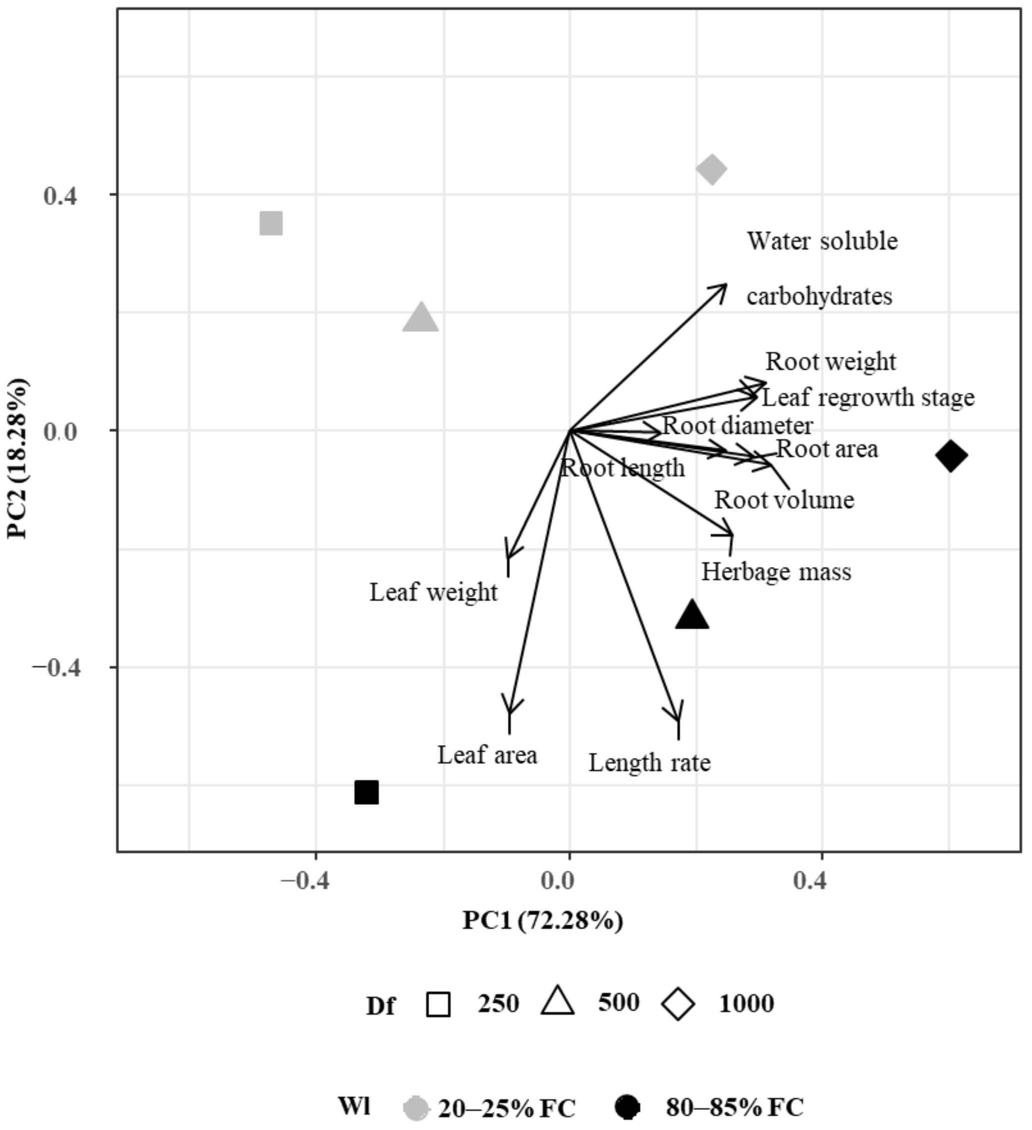


Figure 3.2. Principal component analysis showing changes in the growth variables and their relationship for *Bromus valdivianus* Phil. due to defoliation frequency (Df) based on accumulated growing degree days: 250 (high) (□), 500 (medium) (△) and 1000 (low) (◇) accumulated growing degree days, and two levels of soil-water restriction (Wl): 80–85% field capacity (FC) (shaded black) and 20–25% FC (shaded grey).

3.5 DISCUSSION

This glasshouse study highlighted the effect of defoliation frequency on Bv plant growth and structure. Defoliation frequency during a period of soil-water scarcity has previously been shown to have a significant effect in terms of plant fitness to survive and recovery (Fulkerson and Donaghy, 2001). Results of the WSC reserve status supported the optimal LS range (3–4) suggested by Ordoñez et al. (2017) and provided further evidence for managing defoliation frequency in accordance with LS criteria. In a study of defoliation frequency effects based on LS for different grass species, Turner et al. (2006) showed the importance of species-specific defoliation intervals. Poor productivity and persistence of Bv in field experiments in New Zealand (Fasi et al., 2008; Moot et al., 2019) could be a consequence of defoliation management based on other species, e.g., perennial ryegrass.

The finding that infrequent defoliation allowed a greater tiller weight and thus a greater WSC content (Figure 3.1) indicates how the priority of energy allocation in the plant changes during regrowth after defoliation, which supports earlier findings by Danckwerts and Gordon (1987). Donaghy and Fulkerson (1998) stated that leaf regrowth in perennial grasses has the highest priority for WSC reserves immediately after defoliation and the current study appears to support of this rationale as the high frequency treatment, which corresponded with 1.5 LS, resulted in a higher leaf area rate and SLA rate per tiller compared with infrequent defoliations (Figure 3.2). However, this relationship could also have been an adaptive strategy by Bv plants subjected to more frequent defoliation, to apportion more WSC to leaf regrowth.

The result of a larger root mass at the low defoliation frequency is also in agreement with the study by Donaghy and Fulkerson (1998) on *Lolium perenne* L., where the regrowth of roots coincided with the replenishment of WSC reserves, and greater root mass was associated with less frequent defoliation. Turner et al. (2007) found similar results in *Bromus willdenowii* Kunth., whereby a greater partition of energy to root growth was observed under less frequent defoliation (4 LS). The root system of *B. willdenowii* was also identified as a store of WSC (albeit in lower amounts compared with the stubble) so a greater root mass could also act as a source of WSC for regrowth after defoliation (Johansson, 1993; Turner et al., 2007). If the root system of Bv is similarly an important "secondary" store of WSC, then after a drought period, plants subjected to less frequent defoliation would result in a larger root system and more WSC and these plants would be expected to recover faster than plants with a smaller root system as a result of more frequent defoliation. A fast recovery after drought would also be supported by a greater amount of WSC in the stubble (Figure 3.1; (Volaire et al., 2005)). This interpretation is in accordance with other water stress-tolerant species, such as *D. glomerata*, which increases WSC during droughts, and generates a faster production recovery after re-watering (Volaire et al., 2005).

In the current study, greater total root length, root area and volume measured at medium and low defoliation frequencies compared with high frequency, would be expected to enhance water capture by Bv from deep soil layers under field conditions, allowing plants to avoid dehydration (Norton et al., 2016), and therefore better survive drought conditions. In further support of this, PCA results showed that plant root mass was restricted under 20–25% FC when defoliated at either high or medium defoliation frequencies, compared with plants growing at 20–25% FC and defoliated at the low frequency (Figure 3.2).

In New Zealand, the dry period is in summer, therefore optimal defoliation frequency in late spring is vital to prepare the plants for potential moisture stress conditions. During late spring, grasses display considerable root elongation in deeper soil horizons, while root production declines during summer (Parsons, 1988). Thus, the opportunity to increase soil exploration and root mass is before the summer stress period. However, this strategy would be possible only if the defoliation frequency is at least at a minimum of 3.5 LS (low defoliation frequency), to allow WSC to be allocated to root production.

There was a decrease in LS under soil-water restriction in the current study, due to both slower leaf appearance rate and slower leaf elongation rate, in line with results from studies by Durand et al. (1995), and Bartholomew and Williams (2006). This is further evidence that defoliation management should be optimal prior to the dry period expected in late spring as the ability to manipulate defoliation during a dry period, will be further exacerbated by the direct effect of low moisture levels on LS.

Frequent defoliation was detrimental to the production and energy status of tillers. In the long term, it is expected that this frequent defoliation would lead to an increase in tiller death through depletion of WSC (Fulkerson and Donaghy, 2001), although this was not reflected in a change in tiller number per plant in the current study.

3.6 CONCLUSIONS

The research consistently demonstrated that infrequent defoliations for *Bromus valdivianus* Phil. resulted in plants with a larger root system and greater herbage mass. Despite the lower WSC levels for high defoliation frequency, variables such as tiller number and leaf weight rate per tiller remained unchanged.

Therefore, defoliating *Bromus valdivianus* Phil. at 3.5 leaf regrowth stage increased WSC reserves in the stubble, root mass and herbage mass. *Bromus valdivianus* Phil. pasture should be managed with a defoliation frequency based on 3.5 leaf regrowth stage during late spring in order to ensure that plants have a strong root system prior to the summer dry period. This approach will help enhance summer survival and autumn regrowth.

3.7 REFERENCES

Bartholomew, P., & Williams, R. (2006). Effects of exposure to below-freezing temperatures, soil moisture content and nitrogen application on phyllochron in cool-season grasses. *Grass and Forage Science*, *61*(2), 146-153.

Danckwerts, J., & Gordon, A. (1987). Long-term partitioning, storage and re-mobilization of 14C assimilated by *Lolium perenne* (cv. Melle). *Annals of Botany, 59*(1), 55-66.

Donaghy, D. J., & Fulkerson, W. J. (1998). Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and Forage Science, 53*(3), 211-218.

Donaghy, D. J., Turner, L. R., & Adamczewski, K. A. (2008). Effect of defoliation management on water-soluble carbohydrate energy reserves, dry matter yields, and herbage quality of tall fescue. *Agronomy Journal, 100*(1), 122-127..

Durand, J.-L., Onillon, B., Schnyder, H., & Rademacher, I. (1995). Drought effects on cellular and spatial parameters of leaf growth in tall fescue. *Journal of experimental botany, 46*(9), 1147-1155.

Fasi, V., Mills, A., Moot, D. J., & Pollock, K. (2008). Establishment, annual yield and nitrogen response of eight perennial grasses in a high country environment. *Proceedings of the New Zealand Grassland Association, 70*, 123-130.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture, 41*(2), 261-275.

Harris, W. (1978). Defoliation as a determinant of the growth, persistence and composition of pasture. *Symposium on Plant Relations in Pastures* (pp. 67–85), Brisbane, Qld. (Australia), 15 May 1976, Commonwealth Scientific and Industrial Research Organization.

Johansson, G. (1993). Carbon distribution in grass (*Festuca pratensis* L.) during regrowth after cutting—utilization of stored and newly assimilated carbon. *Plant and Soil, 151*(1), 11-20.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science, 70*(2), 283-295.

McMaster, G. S., & Wilhelm, W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and forest meteorology, 87*(4), 291-300.

Moot, D. J., Smith, M. C., & Mills, A. (2019). Relationships between sheep liveweight production and dry matter yield for lucerne-based pastures in spring. *Journal of New Zealand Grasslands, 81*, 69-74.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of biological chemistry*, *153*(2), 375-380

Norton, M. R., Malinowski, D. P., & Volaire, F. (2016). Plant drought survival under climate change and strategies to improve perennial grasses. A review. *Agronomy for Sustainable Development*, *36*(2), 29..

Ordoñez, I., López, I., Kemp, P., Donaghy, D., Herrmann, P., Hernández, F., & Bhatia, S. (2017). Pasture brome (*Bromus valdivianus*) leaf growth physiology: a six-leaf grass species. *Agronomy New Zealand*, *47*, 13-22.

Parsons, A. (1988). The effects of season and management on the growth of grass swards. In *The grass crop* (pp. 129-177). Springer, Dordrecht.

Team, R.C. (2013). R: A language and environment for statistical computing.

Thomas, H., & James, A. R. (1999). Partitioning of sugars in *Lolium perenne* (perennial ryegrass) during drought and on rewatering. *The New Phytologist*, *142*(2), 295-305.

Tsimba, R., Edmeades, G. O., Millner, J. P., & Kemp, P. D. (2013). The effect of planting date on maize: Phenology, thermal time durations and growth rates in a cool temperate climate. *Field Crops Research, 150*, 135-144.

Turner, L., Donaghy, D., Lane, P., & Rawnsley, R. (2007). Patterns of leaf and root regrowth, and allocation of water-soluble carbohydrate reserves following defoliation of plants of prairie grass (*Bromus willdenowii* Kunth.). *Grass and Forage Science, 62*(4), 497-506.

Turner, L. R., Donaghy, D. J., Lane, P. A., & Rawnsley, R. P. (2006). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 1. Regrowth, tillering and water-soluble carbohydrate concentration. *Grass and Forage Science*, *61*(2), 164-174.

Turner, N. (1986). Adaptation to water deficits: a changing perspective. *Functional Plant Biology*, *13*(1), 175-190.

Volaire, F., Morvan-Bertrand, A., Prud'homme, M. P., Benot, M.L., Augusti, A., Zwicke, M., Roy, J., Landais, D., & Picon-Cochard, C. (2020). The resilience of perennial grasses under two climate scenarios is correlated with carbohydrate metabolism in meristems. *Journal of experimental botany*, *71*(1), 370-385.

Volaire, F., Norton, M. R., Norton, G. M., & Lelievre F. (2005). Seasonal patterns of growth, dehydrins and water-soluble carbohydrates in genotypes of *Dactylis glomerata* varying in summer dormancy. *Annals of botany*, *95*(6), 981-990.

CHAPTER 4

Decreasing defoliation frequency enhances *Bromus valdivianus* growth under low soil water levels and interspecific competition

Competition results from the reaction of one plant to physical process between limited factors and competitors. While in Chapter 3 the optimum defoliation frequency to enhance *Bromus valdivianus* growth was defined, the objective of the present chapter was to assess competition effect from *L. perenne* on *B. valdivianus* growth. This was evaluated under different defoliation frequencies and two soil water availabilities.

Agronomy, Published. Reference: García-Favre, J., Zhang, Y., López, I. F., Donaghy, D. J., Cranston, L. M., & Kemp, P. D. (2021). Decreasing Defoliation Frequency Enhances *Bromus valdivianus* Phil. Growth under Low Soil Water Levels and Interspecific Competition. *Agronomy*, *11*(7), 1333.

4.1 ABSTRACT

Bromus valdivianus Phil. (Bv) is a water stress-tolerant species, but its competitiveness in a diverse pasture may depend on defoliation management and soil moisture levels. This glasshouse study examined the effect of three defoliation frequencies, based on accumulated growing degree days (AGDD) (250, 500, and 1000 AGDD), and two soil water levels (80–85% of field capacity (FC) and 20–25% FC) on Bv growth as monoculture and as a mixture with *Lolium perenne* L. (Lp). The treatments were applied in a completely randomised block design with four blocks. The above-ground biomass of Bv was lower in the mixture than in the monoculture ($p \leq 0.001$). The Bv plants in the mixture defoliated infrequently (1000 AGDD) showed an increase in root biomass under 20–25% FC compared to 80–85% FC, with no differences measured between soil water levels in the monoculture. Total root length was higher in the mixture when infrequent defoliation and under 20–25% FC. Conversely, frequent defoliation treatments resulted in reduced water-soluble carbohydrate reserves in the tiller bases of plants ($p \leq 0.001$), as they allocated assimilates mainly to foliage growth. These results provide evidence that *B. valdivianus* is more competitiveness relative to Lp through the enhancement of root growth and energy reserve in the tiller base under moisture stress when infrequent defoliated.

4.2 INTRODUCTION

Natural grasslands and anthropogenic pastures cover 31–43% of Earth’s terrestrial habitats (Gibson and Newman, 2019). Grasslands are composed of different functional types of species (e.g., woody, graminoid, forb), where the species diversity is closely linked to climate and herbivory history in the ecosystem (Diaz et al., 2007). In New Zealand, *Lolium perenne* L. (Lp) and *Trifolium repens* L. dominate seed sales and are widely used as mixed pastures. Although management practices (i.e., defoliation frequency, nutrient applications) for this simple mixture are well understood by farmers in New Zealand, Hooper et al. (2005) have reported that enhancing pasture diversity can improve herbage mass production. A greater herbage mass production of mixed pastures is based on the plant functional diversity of the ecosystem (Sanderson, 2010; Tilman, 2001), that is, how the ecosystem functionality and stability are modified by species richness and thus species traits (Proulx et al., 2010).

Morphological plant traits, such as root depth and specific leaf area (SLA) indicate how a plant behaves and, thus, how it interacts in the ecosystem (i.e., competes) with neighbours (Grime, 1977). These traits build up plant growth strategies and can be classified as fast traits and slow traits (Reich, 2014). Fast traits are found in plants that are well adapted to rich environments and consist of fast above and below ground growth rates that provide strong competition for resources (Fort et al., 2013). They are associated with a dehydration avoidance strategy (Turner, 1986) and a low survival under extreme droughts (Reich, 2014). On the other hand, plants with slow traits invest more resources to acquire a deep and coarse root system to maintain a minimum of water uptake, even during extreme droughts (Fort et al., 2013), which confers a dehydration tolerance strategy to the species (Turner, 1986).

Lolium perenne is classified as a species with fast traits and a highly competitive strategy (Grime et al., 2014). As a result, in New Zealand, under favourable environmental conditions, the accumulated herbage mass of Lp can reach 16.5 to 18 t (dry matter) DM year^{-1} under grazing (Clement et al., 2016). In more stressful environments, such as New Zealand's hill country, which can have steep slopes and shallow soils with a limited soil water holding capacity (Kemp & López, 2016), Lp growth rate is reduced (Hofer et al., 2016), and Lp is outperformed by species with lesser foraging potential (i.e., low forage quality, such as *Agrostis capillaris* L.), but that are better suited to those conditions (Kemp & López, 2016).

The increasing frequency and severity of summer droughts in some regions of New Zealand (e.g., eastern regions) as a consequence of climate change (Henessy et al., 2007) have shifted the growth and conditions of pastures from high growth rate and high-quality herbage to low growth rates and quality within the same year. The consequence of this in rainfed systems is a high tiller death of Lp during summer droughts, which reduces the annual production and persistence of Lp (Woodward et al., 2020). Therefore, under these conditions, the ecological niche left empty by species such as Lp could be suitable for other pasture species belonging to a different functional group, such as more water stress-tolerant species (Tilman, 2001).

Previous studies have shown how Lp competes for resources and how it performs in a mixture and as a monoculture under contrasting environments (Teughels et al., 1995; Zhao et al., 2017). In these studies, the growth of *Festuca arundinacea* L. (the accompanying species) was suppressed by Lp under different environmental constraints (i.e., short drought, high temperatures). However, due to the effects of climate change and the increasing reduction in Lp plant survival under severe drought, the study of other desirable species is necessary. *Bromus valdivianus* Phil. (Bv) is a grass species native to the south of Chile (temperate humid climate, similar to New Zealand), with similar soil physical and fertility requirements to Lp (López et al., 1997). Although it is commercially available to farmers, and it produces a similar herbage mass quantity and quality as Lp (Calvache et al., 2020; Ramírez et al., 2014), it is not widely used in New Zealand. Unlike Lp, Bv has a slow trait strategy (i.e., deep root system), that allows it to maintain growth and compete better for resources during the warm season, when low soil water conditions negatively affect the herbage mass production (Keim et al., 2015; López et al., 2013). In addition, Ordoñez et al. (2017) reported that Bv can maintain up to six leaves per tiller, while López et al. (2013) stated that Bv has a bigger tiller size compared to Lp, but with a lower tiller number per unit area. It was also reported that these species can coexist in permanent grasslands in the south of Chile (López et al., 1997), and further research described the competition processes between Bv and Lp (López et al., 2013).

Pasture defoliation frequency is a species-specific criterion and determines pasture production and persistence (Turner et al., 2006b). This criterion is based on the replenishment of water-soluble carbohydrates (WSC) in the base of tillers prior to a pasture defoliation event (Fulkerson and Donaghy, 2001). Leaf regrowth stage (number of leaves per tiller) is a morphological parameter closely related to WSC levels in the tiller base, as well as to forage quality and DM yield (Fulkerson and Donaghy, 2001; Turner et al., 2006a). Very little is known about how defoliation frequency affects species production and persistence in a mixture of two grasses differing in their functional strategy (Bv and Lp in this case). In addition, little is known regarding how changes in defoliation frequency (i.e., a higher defoliation frequency) may benefit the growth of slow trait strategy species under contrasting soil water conditions (high or low soil water levels).

The abundance and persistence of species in a mixed pasture depends on the interaction between the habitat, the strategies and competition process between species (Harris et al., 1981). A clear understanding of these processes, which are related to changes in morphological traits in the species, along with optimal defoliation practices to secure species persistence, are essential to successfully incorporating new species into a farming system (Sanderson et al., 2004; Tow and Lazenby, 2000). It is important to determine: (a) how interspecific and intraspecific competition shift under contrasting soil water conditions, (b) how Bv morpho-physiology grown in a mixture with Lp is modified, and (c) how defoliation frequency impacts species persistence and promotes species functional traits. Therefore, it was hypothesised that a mixed pasture of a slow trait strategy (Bv) and a fast trait strategy (Lp) species presents a greater growth of the former compared with its monoculture under low available soil water levels. However, under well-watered conditions, Lp will compete strongly for resources (nutrients, light) and potentially outcompete Bv. Thus, the main objective of the current study was to identify and analyse the main morphological and physiological changes in Bv mixed with Lp, compared to a Bv monoculture, under contrasting defoliation frequencies and two soil water conditions.

4.3 MATERIALS AND METHODS

4.3.1 Location and Experimental Design

The study was carried out in a glasshouse at Massey University’s Plant Growth Unit, (40.37° latitude south and 175.61° longitude west), from 9 October 2018 to 12 March 2019. A total of 204 pots, each of 8 L capacity (24 cm upper diameter, 17 cm lower diameter and 24 cm height) were filled with a mixture of 30% Manawatu silt loam soil and 70% fine sand to achieve a sandy loam texture. On 9 October, in each pot, 2 seeds of either *Bromus valdivianus* Phil. cv. Bareno (Bv) or *Lolium perenne* L. cv. Trojan (Lp) were planted in 24 equidistance positions (i.e., totalling 24 plants per pot), with 40 mm between them.

At the early seedling establishment stage, the number of plants per pot was thinned to one plant (of either Bv or Lp) per position. A combination of two types of pastures, three defoliation frequencies and two soil water restriction levels (2 × 3 × 2 factorial arrangement; 12 treatments) were applied in a complete randomised block design with four blocks (four pots per treatment). Two pasture types were sown: (1) a monoculture of Bv; and (2) a mixture of 50% Bv and 50% Lp. In the mixture, both species competed for space and resources. The monoculture pasture was the control to capture any physiological and morphological growth adjustments in Bv due to the presence of Lp.

In order to produce the six live leaves that each tiller maintains, Bv required 1000 accumulated growing degree days (AGDD), based on the results of Ordoñez et al. (2017). The temperature inside the glasshouse was recorded daily at 10 min intervals and AGDD was calculated following the method of McMaster and Wilhelm (1997), using 0 °C as base temperature. For the whole experimental period, the average temperature was 22.5 °C, while the maximum and minimum temperature were 27.8 °C and 17.2 °C, respectively. Thus, three defoliation frequencies were applied: 250, 500 and 1000 AGDD. The 250 AGDD treatment evaluated pasture growth under a highly intensive defoliation regimen (defoliated four times during each 1000 AGGD period), while the 500 AGDD treatment was an intermediate defoliation frequency (defoliated two times).

In addition, two soil water levels were applied as follows: 80–85% of field capacity (FC) and 20–25% FC. The 80–85% FC was the control for the soil water restriction treatment. The volumetric water contents were continuously monitored by ECH2 O EC-5 soil moisture sensors (METER Group, Inc., Pullman, WA, USA) located in the soil at the centre of 12 randomly selected pots (one pot per treatment combination), which recorded data every 15 min. The soil moisture levels in each treatment were readjusted daily by irrigation according to the following formula:

$$I = ([IC - WC]/100) \times BD \times SD \times PA$$

where I: irrigation (L m^{-2}); IC: irrigation criteria (vol. %); WC: substrate water content (vol. %); BD: bulk density (mg m^{-3}); SD: substrate depth (m); PA: pot area (0.045 m^2).

In order to obtain the water release curve of the substrate, samples were taken from six supernumerary pots using one cylinder (volume: 147 cm3) per pot and applying them to a pressure plate apparatus at different pressures. Field capacity was reached at a pressure of 60 hPa and 16% of the volume of the substrate, and permanent wilting point (PWP) at a pressure of 15,000 hPa and 2% of the volume of the substrate.

In addition, 13 extra repetitions were managed under the same conditions as the corresponding pots to each treatment. These “spare” pots were utilised at the final harvest to increase the sample amount in physiological and morphological determinations (explained below).

The results of the chemical analysis of the substrate were as follows: pH 6.3 (1:2 (soil:water)), 35 mg L^{-1} Olsen phosphorus, 0.34 me 100 g^{-1} potassium, 2.4 me 100 g^{-1} calcium, 0.6 me 100 g^{-1} magnesium, 3 me 100 g^{-1} cation exchange capacity and 83 mg kg^{-1} sulfate sulfur. Based on this analysis, the following fertiliser was added to each 60 L of substrate: 120 g of slow-release formula (14% nitrogen (N), 5% phosphorus, 10% soluble potash, 0.5% magnesium, 3.2% sulfur, 1.6% iron and 0.3% manganese), 60 g short term formula (14% total N, 6% phosphorus, 11.6% potassium, 1% magnesium, 4% sulphur, 1% iron and 0.5% manganese), and 90 g of dolomite (calcium-carbonate).

4.3.2 Experimental Stages

The length of the plant establishment period was 9 weeks, which allowed plants to produce roots and at least three tillers per plant before the beginning of the experimental stages. At the end of the establishment phase (when the 6th Bv leaf appeared in the older tillers), all plants were defoliated, and two plants located in the centre of each pot were marked at their base with a paper clip. This indicated the start of the defoliation frequency treatments. All defoliations were performed to a 50 mm height. The plants were managed without any water restriction for 1000 AGDD (when the most infrequent defoliation treatment was reached); at this stage, the 500 AGGD defoliation treatment had been defoliated twice and the 250 AGGD had been defoliated four times. This period was utilised as a pre-treatment stage to allow the plants to adapt their architecture to the defoliation treatment received. After this, the soil water treatments were imposed and plants completed a further 1000 AGDD, including their associated defoliation frequency treatments (all the measurements occurred at this stage).

4.3.3 Measurements

Two tillers per pot were marked with paper clips at their base, and tiller leaf length and number of leaves per tiller were recorded every three days throughout the stage. For the 250 and 500 AGDD treatments, the average leaf length of four and two growth cycles, respectively, was used. The leaf length of an elongating leaf included the distance from the ligule of the previous fully expanded leaf to the lamina tip, and for a fully expanded leaf considered the distance from the ligule to its tip. Tiller density was monitored once a week by counting the number of tillers in the two central plants per pot. At the final harvest, the two marked tillers were cut to ground level and the following measurements were made: leaf regrowth stage (LS) as the number of fully expanded leaves per tiller, leaf area tiller^{-1} (mm^{2} °Cd^{-1}) using a leaf area meter (LI-COR 3100, area meter), SLA (mm^2 mg °Cd^{-1}) as the quotient between leaf area (mm2 °Cd^{-1}) and leaf weight (mg °Cd^{-1}) by drying the leaves at 70 °C for 48 h in an air forced oven. Each variable was then divided by the time elapsed in each defoliation frequency, to calculate their rate. The remaining plants in each pot were cut to 50 mm. The stubble (tiller base below the 50 mm defoliation height) was cut at ground level and frozen with liquid nitrogen before freeze-drying. Then, the samples were weighed and analysed for WSC and starch content by the Nutritional Laboratory, Massey University (Palmerston North, New Zealand). The WSC was determined using the colorimetric assays developed by Somogyi-Nelson (Nelson, 1944). Starch content was quantified using a modified Megazyme protocol (Megazyme Total Starch Assay Procedure, AOAC method 996.11, Megazyme International, Ireland). All harvests were undertaken between 9 and 11 am to avoid diurnal changes in WSC (Fulkerson and Slack, 1995).

In three “spare” pots per treatment, one centre plant was selected and harvested and separated into above ground from below ground biomass. Above ground biomass was weighed after being dried at 70 °C for 48 h. The roots were washed using a 1 mm sieve, scanned with an EPSON scanner (400 dpi) to enable total root length, root surface area, root volume and root diameter to be analysed using Winrhizo software (ver. 2012b, Regent Instruments Inc., Quebec, QC, Canada). After that, root biomass per plant was obtained following the same procedure as above ground biomass, and root mass fraction was calculated as the ratio of root mass to total mass. The rest of the pots were used to measure malondialdehyde (MDA) concentration. The MDA concentration in the last full expanded leaf in a tiller was determined by the thiobarbituric acid reaction as follows (Hodges et al., 1999): 0.5 g of green leaf material was collected and frozen immediately using liquid nitrogen. In a laboratory, each sample had 5 mL of 5% trichloroacetic acid added to it. The mixture was ground and centrifuged at 3000 rpm for 10 min. After that, 2 mL of the supernatant obtained was added to 2 mL of 0.67% thiobarbituric acid and incubated in a boiling water bath for 30 min, and then centrifuged. The supernatant was displaced in a spectrometer and used the following formula to obtain the concentration:

$$\text{MDA } (\mu\text{mol L}^{-1}) = 6.45(\text{A}532 - \text{A}600) - 0.56\text{A}440$$

where A is the absorbance value at different wavelengths (532, 600 and 440 nm).

4.3.4 Statistical Analysis

The statistical model for the analyses performed at the tiller and plant level evaluated two classes of competition treatments (individual tillers and plants of Bv grown in a monoculture or Bv tillers and plants grown in a mixture (50% Bv–50% Lp)). Both treatments were subjected to three defoliation frequencies (250 AGDD, 500 AGDD and 1000 AGDD) and two soil water restriction levels (80–85% FC and 20–25% FC). Thus, the model performed the individual interactions between treatments and the triple interaction (competition level–defoliation frequency–soil water restriction level). R statistical software was used to perform a one-way analysis of variance (ANOVA), least significant difference (LSD) and canonical variate analysis (CVA), to analyse statistical differences and relationships between treatments and measured variables, with a level of significance of 5% (p = 0.05) (Team, 2013). The CVA analysis was performed with Candisc package and the biplot was performed with Heplot package only for the morphological traits. More details about CVA analysis can be found in López et al. (2003)

4.4 RESULTS

4.4.1 Tiller Components

In the mixture, the leaf area and the tiller weight of Bv diminished by 20% ($p \leq 0.05$). The 20–25% FC treatments diminished leaf area (30%) ($p \leq 0.001$), SLA (33%) ($p \leq 0.01$), lamina length rate (52%) ($p \leq 0.01$) and accumulated leaf length (51%) ($p \leq 0.01$)

of Bv. Conversely, leaf weight per plant was not affected by soil water levels ($p \geq 0.05$) (Table 4.1).

Leaf area, leaf weight and SLA per tiller of Bv increased under the most frequent defoliation ($p \leq 0.01$), while accumulated leaf length per tiller showed the lowest value at this defoliation frequency (Table 4.1). Lamina length rate increased under the 1000 AGDD compared to the 250 AGDD treatment ($p \leq 0.001$), but there was no significant difference ($p \geq 0.05$) between the 1000 and 500 AGDD treatments. The interaction between defoliation frequency and soil water level was only significant for LS ($p \leq 0.01$). The number of leaves increased with decreasing defoliation frequency and under well-watered conditions ($p \leq 0.001$). Conversely, tillers per plant did not show any modification by any of the factors ($p \geq 0.05$) (Table 4.1). The defoliation frequency and pasture type interaction, pasture type and water level interaction, and three-way interaction between main factors were not significant ($p > 0.05$).

Table 4.1. Tiller components of *Bromus valdivianus* (Bv) grown under two pasture types (monoculture and in a 50/50% mixture with *Lolium perenne*), and subjected to three levels of defoliation frequency (250, 500 and 1000 accumulated growing degree days (AGDD)), and two levels of soil water content (80–85% and 20–25% of field capacity (FC)). Values are presented as mean ± SEM.

	Leaf Area/Tiller (mm^2 °Cd^{-1})	Leaf Weight/Tiller (mg °Cd^{-1})	Leaf Regrowth Stage	Specific Leaf Area (mm^2 mg^{-1} °Cd^{-1})	Lamina Length Rate (mm °Cd^{-1})	Accumulated Leaf Length (mm)	Tillers No. plant^{-1}
Pasture type							
Bv Monoculture	1.86 ± 0.14 a	0.15 ± 0.01 a	2.04 ± 0.17 a	0.03 ± 0.01	0.34 ± 0.03	258.79 ± 21.14	4.38 ± 0.25
Bv Mixture	1.49 ± 0.15 b	0.12 ± 0.01 b	1.80 ± 0.18 b	0.03 ± 0.01	0.34 ± 0.03	258.44 ± 24.65	3.92 ± 0.28
Significance	*	*	*	ns	ns	ns	ns
Defoliation frequency (AGDD)							
250	2.13 ± 0.17 a	0.16 ± 0.01 a	1.15 ± 0.10 c	0.05 ± 0.01 a	0.30 ± 0.03 b	210.53 ± 23.56	4.28 ± 0.32
500	1.60 ± 0.92 b	0.12 ± 0.01 b	1.71 ± 0.08 b	0.03 ± 0.01 b	0.34 ± 0.03 ab	290.31 ± 27.94	4.00 ± 0.31
1000	1.30 ± 0.11 b	0.12 ± 0.01 b	2.84 ± 0.16 a	0.01 ± 0.01 c	0.39 ± 0.04 a	275.00 ± 29.17	4.16 ± 0.36
Significance	***	**	***	***	**	**	ns
Water level							
20–25 % FC	1.39 ± 0.11 b	0.13 ± 0.01	1.61 ± 0.13 b	0.02 ± 0.01 b	0.22 ± 0.02 b	170.13 ± 12.95	4.21 ± 0.25
80–85 % FC	1.96 ± 0.16 a	0.14 ± 0.01	2.20 ± 0.19 a	0.03 ± 0.01 a	0.46 ± 0.02 a	347.10 ± 14.35	4.08 ± 0.28
Significance	***	ns	***	**	***	***	ns
Defoliation frequency × Water level							
250 × 20–25 % FC	1.74 ± 0.08	0.15 ± 0.01	0.95 ± 0.14 e	0.05 ± 0.01	0.18 ± 0.02	131.38 ± 14.26	4.25 ± 0.57
250 × 80–85 % FC	2.52 ± 0.26	0.18 ± 0.01	1.33 ± 0.12 d	0.06 ± 0.01	0.41 ± 0.03	289.69 ± 9.62	4.17 ± 0.59
500 × 20–25 % FC	1.35 ± 0.26	0.12 ± 0.02	1.53 ± 0.10 d	0.02 ± 0.01	0.24 ± 0.03	204.13 ± 28.03	3.58 ± 0.20
500 × 80–85 % FC	1.84 ± 0.24	0.12 ± 0.02	1.89 ± 0.08 c	0.04 ± 0.01	0.44 ± 0.02	376.50 ± 20.93	4.08 ± 0.49
1000 × 20–25 % FC	1.08 ± 0.08	0.12 ± 0.01	2.28 ± 0.09 b	0.01 ± 0.01	0.25 ± 0.02	174.88 ± 16.80	4.58 ± 0.78
1000 × 80–85 % FC	1.52 ± 0.17	0.12 ± 0.02	3.39 ± 0.13 a	0.01 ± 0.01	0.53 ± 0.03	375.13 ± 22.35	3.33 ± 0.44
Significance	ns	ns	**	ns	ns	ns	ns

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

4.4.2 Plant Morphological Traits at Final Harvest

At the final harvest, the interaction between pasture type and soil water level was significant, except for root diameter, for all of the evaluated variables at plant level (Table

4.2). The 80–85% FC treatments provided the highest values for Bv above ground biomass, root biomass, and root volume in the monoculture. In contrast, under 20–25% FC, there was no significant difference ($p \geq 0.05$) in the total root length and root area of Bv plants grown in monoculture or mixture. Root diameter was only affected by pasture type, with an increase in Bv plants growing in the monoculture ($p \leq 0.01$).

A reduction in the defoliation frequency increased above ground biomass, root biomass, root area, and root volume ($p \leq 0.001$). Total root length showed an interaction ($p \leq 0.05$) between defoliation frequency and soil water level, where Bv under the most infrequent defoliation and 20–25% FC had the longest root system (Table 4.2). The defoliation frequency and pasture type interaction, and three-way interaction between main factors were not significant ($p > 0.05$).

Table 4.2. Above and below ground components of *Bromus valdivianus* (Bv) plants grown under two pasture types (monoculture and in a 50/50% mixture with *Lolium perenne*), and subjected to three levels of defoliation frequency (250, 500 and 1000 accumulated growing degree days (AGDD)), and two levels of soil water content (80–85% and 20–25% of field capacity (FC)). Values are presented as mean ± SEM.

	Above Ground Biomass (g DM plant^{-1})	Root Biomass (g DM plant^{-1})	Total Root Length (cm)	Root Area (cm^2)	Root Diameter (mm)	Root Volume (cm^3)
Pasture type						
Bv Monoculture	1.06 ± 0.19 a	0.36 ± 0.05 a	2589.15 ± 374.82	204.73 ± 28.51	0.23 ± 0.01 a	1.18 ± 0.18
Bv Mixture	0.47 ± 0.06 b	0.25 ± 0.04 b	3135.07 ± 469.16	191.83 ± 29.42	0.20 ± 0.01 b	0.96 ± 0.16
Significance	***	**	ns	ns	**	ns
Defoliation frequency (AGDD)						
250	0.38 ± 0.07 b	0.14 ± 0.02 c	1679.95 ± 191.45 c	100.00 ± 10.91 c	0.19 ± 0.01	0.49 ± 0.06 c
500	0.85 ± 0.20 a	0.29 ± 0.04 b	2698.91 ± 358.41 b	183.97 ± 27.05 b	0.22 ± 0.01	1.02 ± 0.17 b
1000	1.05 ± 0.19 a	0.50 ± 0.05 a	4612.46 ± 522.49 a	310.87 ± 31.64 a	0.22 ± 0.01	1.71 ± 0.17 a
Significance	***	***	***	***	ns	***
Water level						
20–25 % FC	0.67 ± 0.10	0.30 ± 0.05	3018.90 ± 512.45	187.19 ± 31.29	0.20 ± 0.01	0.94 ± 0.16
80–85 % FC	0.86 ± 0.16	0.32 ± 0.04	2975.32 ± 316.51	209.37 ± 26.25	0.22 ± 0.01	1.20 ± 0.17
Significance	ns	ns	ns	ns	ns	ns
Pasture type × Water level						
Bv Monoculture × 20−25 FC	0.80 ± 0.17 b	0.31 ± 0.06 b	2280.40 ± 457.28 c	156.07 ± 30.85 b	0.22 ± 0.01	0.86 ± 0.17 b
Bv Monoculture × 80−85 FC	1.32 ± 0.24 a	0.42 ± 0.07 a	3437.90 ± 551.58 ab	253.39 ± 43.71 a	0.23 ± 0.01	1.51 ± 0.28 a
Bv Mixture × 20−25 FC	0.54 ± 0.10 bc	0.29 ± 0.08 b	3757.40 ± 877.86 a	218.13 ± 54.48 ab	0.19 ± 0.01	1.02 ± 0.27 b
Bv Mixture × 80−85 FC	0.39 ± 0.06 c	0.22 ± 0.04 b	2515.74 ± 260.81 bc	165.36 ± 23.11 b	0.21 ± 0.01	0.89 ± 0.16 b
Significance	*	**	**	**	ns	**
Defoliation frequency × Water level						
250 × 20–25 FC	0.28 ± 0.06	0.11 ± 0.04	1421.85 ± 262.75 d	81.67 ± 10.52	0.19 ± 0.01	0.38 ± 0.04
250 × 80–85 FC	0.49 ± 0.10	0.17 ± 0.02	1938.04 ± 256.10 cd	118.33 ± 16.70	0.20 ± 0.01	0.59 ± 0.10
500 × 20–25 FC	0.61 ± 0.03	0.25 ± 0.03	2275.26 ± 478.84 cd	144.87 ± 25.70	0.21 ± 0.01	0.74 ± 0.11
500 × 80–85 FC	1.10 ± 0.38	0.33 ± 0.08	3122.56 ± 513.95 bc	223.08 ± 44.14	0.22 ± 0.02	1.30 ± 0.30
1000 × 20–25 FC	1.12 ± 0.18	0.54 ± 0.05	5359.58 ± 814.73 a	335.02 ± 47.56	0.21 ± 0.01	1.69 ± 0.24
1000 × 80–85 FC	0.98 ± 0.28	0.45 ± 0.08	3865.35 ± 560.47 b	286.72 ± 43.71	0.24 ± 0.01	1.72 ± 0.71
Significance	ns	ns	*	ns	ns	ns

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$; ns, not significant ($p > 0.05$).

4.4.3 Effects on Plant Growth Morphology under Different Defoliation Frequencies

Overall, Bv plants in the mixture and defoliated at 1000 AGDD increased root biomass, total root length and root mass fraction when the soil water level changed from to 80–85% FC to 20–25% FC (Figure 4.1). As a result, under 20–25% FC, total root length and root biomass fraction of Bv in the mixture was greater than Bv plants in monoculture when defoliated at 1000 AGDD. Conversely, these variables of Bv in the monoculture did not change when the soil water level declined, except for root volume which decreased. Furthermore, above ground biomass showed, for both water levels, a higher value in the monoculture than in the mixture; and in the mixture it increased under 20–25% FC. Root volume did not change between pasture types (Figure 4.1).

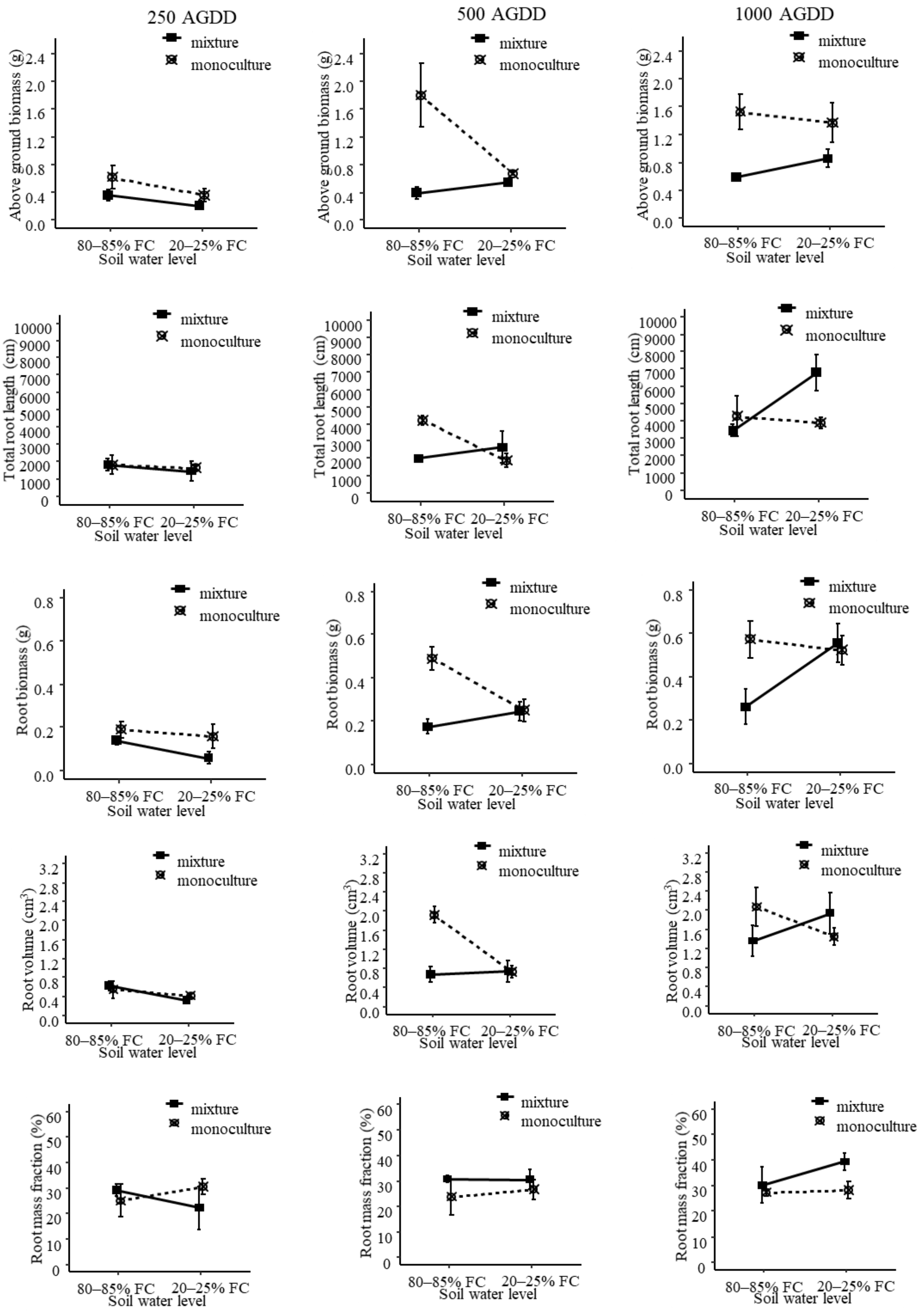


Figure 4.1. Effect of variation in soil water content, from 80–85% of field capacity (FC) to 20–25% FC on *Bromus valdivianus* morphology grown under two pasture types (monoculture and in a 50/50% mixture with *Lolium perenne*), and subjected to three levels of defoliation frequency (250, 500 and 1000 accumulated growing degree days

(AGDD)). The first column shows 250 AGDD defoliation treatment, the second 500 AGDD ad the third 1000 AGDD treatment.

With the 500 AGDD defoliation treatment, Bv plants in the monoculture under 80–85% FC had higher values in above ground biomass, total root length, root biomass and root volume than Bv in the mixture (Figure 4.1). However, these variables decreased in Bv in the monoculture and under soil water restrictions, with no differences between pasture types under 20–25% FC. The Bv in the mixture did not vary in any of the measured variables when soil water level was modified. Root mass fraction did not show differences under any of the treatments.

Under the most frequent defoliation (250 AGDD), the root biomass of Bv in the mixture decreased under 20–25% FC. Further, plants in the monoculture under 20–25% FC had greater root biomass than plants in the mixture (Figure 4.1). Above ground biomass was higher in the monoculture under well-watered conditions, but decreased with soil water restriction, to a similar amount as the above ground biomass in the mixture. Total root length, root volume and root mass fraction did not show any differences between pasture types and soil water levels.

The CVA explained 93.8% of the total differences between the treatments with a significant Wilks' lambda ($p \leq 0.001$); the first two canonical variates explained 93.8% of the differences between groups (CAN 1 = 79.9%, $p \leq 0.001$; CAN 2 = 13.9%, $p \leq 0.001$) (Figure 4.2). Along CAN 1 the treatments were separated by defoliation frequency, in a positive direction, such that treatments under 250 AGDD defoliation were related with high SLA values. In the other extreme of CAN 1, the treatments under the most infrequent defoliation were located and the variables associated with them were related to the increase in root size. Along CAN 2, treatments were separated by soil water level, such that increasing leaf size under 80–85% FC conditions was located in the positive direction, while increasing root length was strongly associated with 20–25% FC in the opposite direction.

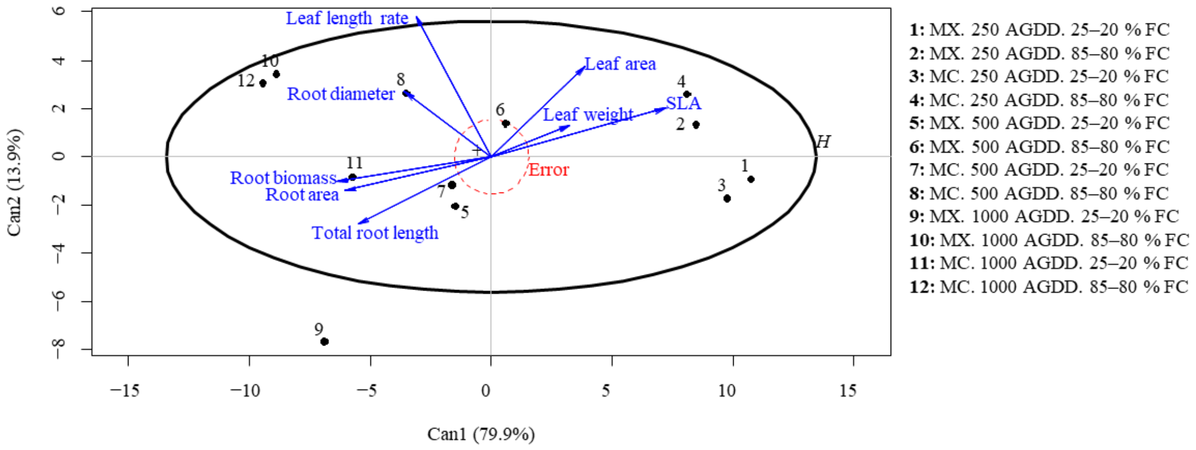


Figure 4.2. Changes in morphological variables and their relationships for *Bromus valdivianus* grown under two pasture types (monoculture (MC) and in a 50/50% mixture with *Lolium perenne* (MX)), and subjected to three levels of defoliation frequency (250, 500 and 1000 accumulated growing degree days (AGDD)), and two levels of soil water content (80–85% and 20–25% of field capacity (FC)). The size of the null hypothesis (H) ellipsis relative to the error ellipsis (within-group variance) is an indication of the magnitude of the multivariate effect of the group mean.

4.4.4 Changes in Stubble WSC, Starch and Leaf MDA Concentration

The amount of WSC per kg of DM increased ($p \leq 0.001$) with a decrease in soil water level (Table 4.3). The WSC concentration (g kg^{-1} DM) in the stubble under 25–20% FC was 52% and 41% higher for 250 AGD and 500 AGDD treatments, compared with the same defoliation treatments under 80–85% FC, respectively. In the 1000 AGDD defoliation treatment, WSC concentration was at its highest, with higher values for Bv in a monoculture than in a mixture. The WSC content per tiller (mg tiller^{-1}) increased 30% under 25–20% FC ($p \leq 0.001$). Furthermore, there was an interaction between defoliation frequency and pasture type ($p \leq 0.01$), where Bv tillers showed higher content of WSC in the monoculture defoliated at 1000 AGDD, followed by Bv growing in the mixture at the same defoliation frequency. Defoliations at 500 AGDD showed greater WSC amount per tiller than 250 AGDD, with no difference between pasture types (Table 4.3).

Table 4.3. Stubble water-soluble carbohydrate (WSC) content (mg plant^{-1}) and concentration (g kg^{-1} of dry matter, DM), stubble starch content (mg plant^{-1}) and concentration (g kg^{-1} DM) and leaf malondialdehyde (MDA) concentration (µmol L^{-1}) of *Bromus valdivianus* plants grown under two pasture types (monoculture and in a 50/50% mixture with *Lolium perenne*), and subjected to three levels of defoliation frequency (250, 500 and 1000 accumulated growing degree days), and two levels of soil water content (80–85% and 20–25% of field capacity (FC)). Values are presented as mean ± SEM.

	WSC (g kg^{-1} DM)	WSC (mg tiller^{-1})	Starch (g kg^{-1} DM)	Starch (mg tiller^{-1})	MDA (µmol L^{-1})
Pasture type					
Bv Monoculture	58.49 ± 6.49 a	5.54 ± 1.18 a	1.57 ± 0.06	0.14 ± 0.02 a	2.45 ± 0.26
Bv Mixture	52.49 ± 4.95 b	4.17 ± 0.71 b	1.66 ± 0.08	0.11 ± 0.01 b	2.86 ± 0.29
Significance	***	***	ns	***	ns
Defoliation frequency (AGDD)					
250	39.92 ± 4.50 c	1.86 ± 0.30 c	1.50 ± 0.04 b	0.07 ± 0.01 c	3.65 ± 0.6 a
500	44.93 ± 4.11 b	3.07 ± 0.41 b	1.56 ± 0.07 ab	0.10 ± 0.01 b	2.14 ± 0.14 b
1000	83.73 ± 3.51 a	10.00 ± 0.83 a	1.78 ± 0.12 a	0.21 ± 0.02 a	2.35 ± 0.32 b
Significance	***	***	*	***	***
Water level					
20–25% FC	64.59 ± 3.98 a	5.68 ± 0.91 a	1.54 ± 0.05	0.13 ± 0.01	3.12 ± 0.26 a
80–85% FC	46.74 ± 6.31 b	4.04 ± 0.99 b	1.68 ± 0.08	0.12 ± 0.02	2.11 ± 0.25 b
Significance	***	***	ns	ns	***

Continued next page

Defoliation frequency × Water level					
250 × 20–25% FC	53.96 ± 1.85 c	2.78 ± 0.20	1.58 ± 0.07 b	0.08 ± 0.01 d	4.40 ± 0.49
250 × 80–85 % FC	25.87 ± 2.63 e	0.94 ± 0.12	1.41 ± 0.03 b	0.05 ± 0.01 e	2.90 ± 0.24
500 × 20–25% FC	56.43 ± 3.59 c	4.29 ± 0.32	1.49 ± 0.07 b	0.11 ± 0.01 c	2.28 ± 0.25
500 × 80–85% FC	33.44 ± 2.92 d	1.85 ± 0.20	1.63 ± 0.12 b	0.09 ± 0.01 d	2.01 ± 0.16
1000 × 20–25% FC	87.13 ± 3.10 a	10.80 ± 1.14	1.54 ± 0.15 b	0.19 ± 0.03 b	2.88 ± 0.25
1000 × 80–85% FC	80.90 ± 5.94 b	9.34 ± 1.21	1.98 ± 0.13 a	0.22 ± 0.02 a	1.55 ± 0.58
Significance	***	ns	*	***	ns
Pasture type × Defoliation frequency					
Bv Monoculture × 250	41.63 ± 6.38 cd	2.04 ± 0.45 d	1.45 ± 0.06 b	0.07 ± 0.01 d	3.49 ± 0.49
Bv Monoculture × 500	46.07 ± 6.85 c	3.22 ± 0.59 c	1.56 ± 0.08 b	0.11 ± 0.01 c	2.24 ± 0.14
Bv Monoculture × 1000	93.63 ± 1.40 a	12.53 ± 0.70 a	2.02 ± 0.14 a	0.27 ± 0.01 a	1.97 ± 0.45
Bv Mixture × 250	38.20 ± 6.88 d	1.69 ± 0.43 d	1.54 ± 0.06 b	0.06 ± 0.01 d	3.81 ± 0.57
Bv Mixture × 500	43.79 ± 5.19 cd	2.93 ± 0.62 c	1.57 ± 0.12 b	0.10 ± 0.01 c	2.01 ± 0.28
Bv Mixture × 1000	75.49 ± 3.74 b	7.90 ± 0.50 b	1.58 ± 0.14 b	0.16 ± 0.01 b	2.79 ± 0.42
Significance	***	***	*	***	ns
Pasture type × Defoliation frequency × Water level					
Bv Monoculture × 250 × 20–25	55.25 ± 1.83 d	3.01 ± 0.13	1.51 ± 0.11	0.08 ± 0.01	3.95 ± 0.62
Bv Monoculture × 250 × 80–85	28.02 ± 3.87 ef	1.06 ± 0.21	1.39 ± 0.06	0.05 ± 0.01	2.80 ± 0.64
Bv Monoculture × 500 × 20–25	60.55 ± 2.84 cd	4.5 ± 0.25	1.58 ± 0.1	0.12 ± 0.01	2.33 ± 0.16
Bv Monoculture × 500 × 80–85	31.59 ± 4.11 ef	1.93 ± 0.13	1.53 ± 0.14	0.09 ± 0.1	2.16 ± 0.24
Bv Monoculture × 1000 × 20–25	93.89 ± 0.58 a	13.52 ± 0.84	1.83 ± 0.07	0.26 ± 0.01	2.77 ± 0.35
Bv Monoculture × 1000 × 80–85	93.46 ± 2.53 a	11.86 ± 0.92	2.15 ± 0.19	0.27 ± 0.01	0.62 ± 0.03
Bv Mixture × 250 × 20–25	52.68 ± 3.48 d	2.56 ± 0.37	1.65 ± 0.08	0.08 ± 0.01	5.09 ± 0.64
Bv Mixture × 250 × 80–85	23.72 ± 3.85 f	0.81 ± 0.11	1.43 ± 0.02	0.05 ± 0.01	2.96 ± 0.24
Bv Mixture × 500 × 20–25	52.31 ± 6.29 d	4.09 ± 0.65	1.41 ± 0.07	0.11 ± 0.01	2.21 ± 0.74
Bv Mixture × 500 × 80–85	35.28 ± 4.74 e	1.77 ± 0.42	1.73 ± 0.2	0.08 ± 0.01	1.87 ± 0.23
Bv Mixture × 1000 × 20–25	82.63 ± 2.55 b	8.99 ± 0.12	1.35 ± 0.16	0.15 ± 0.02	3.02 ± 0.39
Bv Mixture × 1000 × 80–85	68.35 ± 3.52 c	6.81 ± 0.26	1.81 ± 0.15	0.18 ± 0.01	2.48 ± 0.91
Significance	*	ns	ns	ns	ns

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

There was a significant interaction for starch concentration in the stubble between defoliation frequency and soil water level ($p \leq 0.05$), where the starch concentration increased when Bv was defoliated at 1000 AGDD under well-watered conditions. In addition, there was an interaction between pasture type and defoliation frequency for starch concentration ($p \leq 0.05$) and mg of starch per tiller ($p \leq 0.001$), with the highest value for Bv in the monoculture defoliated at 1000 AGDD (Table 4.3). Defoliation frequency and soil water level interaction was also significant ($p \leq 0.001$), resulting in a decrease in the amount of starch under increased defoliation frequency, while the highest value was reached under 1000 AGDD defoliation and 80–85% FC (Table 4.3).

In addition, MDA concentration in the youngest fully-expanded leaf altered significantly with soil water level ($p \leq 0.001$), with an increase in MDA concentration in plants growing under 20–25% FC. There were also significant differences with defoliation frequency ($p \leq 0.001$), with an increase in MDA concentration in plants defoliated at 250 AGDD compared to less frequent defoliations (Table 4.3). The water and pasture type interaction was not significant ($p > 0.05$).

4.5 DISCUSSION

The current glasshouse study highlighted that the decline in soil water level significantly changed the assimilate allocation pattern to promote soil exploration by Bv roots growing in a mixture. These results support the complementary nature of Bv and Lp sown in a mixture, especially under conditions of soil water restriction, as the species utilise a different ecological niche (i.e., water uptake from different soil layers) and therefore are not regarded as strongly competing for the same resources (Mommer et al., 2010). These results also support the functionality of Bv in the mixture as a water stress-tolerant species.

The increase in Bv total root length under the mixture and 20–25% FC could be the outcome of reduced competition below ground between Bv and Lp. The phenotypic plasticity (i.e., root elongation under 20–25% FC) of Bv plants in the mixture to maximise water uptake was most likely part of the Bv plant strategy to overcome the low soil water availabilities (Chaves et al., 2003). However, this root elongation was only achieved under the most infrequent defoliation, confirming the significant role of defoliation frequency in root development (Turner et al., 2007). This finding agrees with the optimal leaf regrowth stage defoliation frequency for Bv, which Ordoñez et al. (2017) defined as being between 3.5–4.0 LS, to enhance herbage mass production and pasture persistence.

4.5.1 Tiller components

At the tiller level, a lower above ground competitiveness of Bv relative to Lp in the mixture was reflected in a diminishment in Bv leaf area, leaf weight and leaf regrowth stage, which evidenced the difficulty of Bv to capture light when it is competing with a fast trait strategy species, such as Lp. Teughels et al. (1995) reported similar above ground competition between Lp and *Festuca arundinacea* Schreb., with a diminishment in above ground growth of *F. arundinacea*. The leaf area per plant, which is dependent on leaf size and number of leaves per tiller, is the most important component affecting the DM yield of forage species as it plays a key role in capturing light (Chapman and Lemaire, 1993). Therefore, interspecific competition affected Bv foliage mass in the mixture.

The decrease in Bv leaf weight per tiller in the mixture could be related to a decrease in the lamina width, because the accumulated leaf length was not modified by pasture type (Table 4.1). In addition, a lower leaf regrowth stage (number of fully expanded leaves per tiller) in the mixture may have decreased Bv ability to compete for resources with Lp. The leaf regrowth stage is closely related with leaf lifespan in C3 grass species (Gastal and Lemaire, 2015), and could be used as a defoliation frequency criterion in Bv (Ordoñez et al., 2017; Turner et al., 2006b).

The reduction in soil water levels (from 80–85% FC to 20–25% FC) affected Bv tiller development more severely than competition between species, as the leaf regrowth stage was more affected under low soil water conditions (Table 4.1). Soil water constraint triggers different physiological responses in the plant, reducing the length of the division zone and also cell division on the basal part of growing leaves, which decreases leaf appearance and leaf elongation rate (Durand et al., 1995; Durand et al., 1999). These changes affect the phyllochron of grass species and therefore growth under environmental constraint (Bartholomew and Williams, 2006).

The 20–25% FC treatment negatively modified five of the nine measured variables at the tiller level (Table 4.1). As soil water became more limiting, the resource allocation in the plant changed to a more conservative form, by investing less in new leaf appearance and expansion (Mommer et al., 2010). Lastly, the number of tillers per plant was also unaffected by either defoliation frequency or soil water stress, similar to previous studies on Bv (Descalzi et al., 2018; López et al., 2013). This low plant plasticity, keeping all the tillers alive under soil water shortage, has been reported as a plant survival mechanism in some other forage species (Kemp and Culvenor, 2010).

4.5.2 Plant morphological traits at final harvest and effects on the plant growth morphology under different defoliation frequencies

At the final harvest, the above ground biomass results at the plant level were associated with the tiller growth during the study. Intraspecific competition, usually, is higher than interspecific competition due to a lack of niche complementation in the former (Hall, 1978). However, in the present study, above ground biomass in the monoculture was greater than in the mixture under well-watered conditions (Table 4.2). This is similar to results reported by López et al. (2013), who found that Bv above ground biomass was affected by Lp competition under 80–85% FC, 45–50% FC and 20–25% FC. In the present study, root biomass was highest in the mixture and at the most infrequent defoliation under 20–25% FC. Conversely, López et al. (2013) reported the lowest root biomass value under 20–25% FC compared with 80–85% FC and 45–50% FC. The resource use complementarity described by Zhao et al. (2017) under no environmental constraints in a mixture between Lp and *F. arundinacea* also differed from the present results, as *F. arundinacea* root biomass was greater under well-watered conditions. The differences in root mass between the present study and results reported by López et al. (2013) and Zhao et al. (2017) can be explained by the defoliation frequency treatments used in the present study. Under 1000 AGDD defoliation treatment, Bv plants in the mixtures showed an increase in above and below ground biomass when soil water content changed from high to low, with below ground biomass reaching similar values to those of plants in the monoculture (Figure 4.1).

The Bv strategy to maintain a minimum level of water uptake in the mixture and under soil water restriction was confirmed by the increase in total root length and root mass fraction, instead of root volume (Motte et al., 2019). The root length growth of Bv under soil water restriction could have been promoted by hydrotropism catalysed by abscisic acid, probably due to less photosynthetic activity as a consequence of stomatal closure and less shoot biomass available to photosynthesise (Xu and Zhou, 2008). In addition, a lower Bv root diameter in the mixture could be associated with the absolute total root length value of the mixture under 20–25% FC compared to the monoculture, because thinner roots allow a better soil exploration with less investment in root biomass (Bristiel et al., 2018).

The CVA showed that the effect of pasture type was not as strong as defoliation frequency and soil water level effects on the morphology traits of Bv plants. Defoliation frequency was the main driver of change in plant morphology traits, while soil water restriction affected leaf growth and enhanced root elongation on Bv plants subjected to 1000 AGDD defoliation treatment.

In the mixture, only under the combination of the most infrequent defoliation (1000 AGDD) and low soil water levels (20–25% FC) was Bv growth unaffected, while the growth of Bv plants subjected to fast and medium defoliation frequencies was decreased by neighbouring Lp plants. Similarly, López et al. (2013) reported that Lp growth was not affected in a 50/50% mixture of Lp and Bv, compared to Lp in a monoculture. Thus, under field conditions, a decrease in Lp growth should not be expected due to competition from Bv in a mixture.

4.5.3 Changes in stubble water soluble carbohydrate, starch and leaf MDA concentration

The MDA concentration is a result of lipid peroxidation in cell walls due to the attack of active oxygen species that are accumulated in the cells under oxidative stress caused by, for example, high temperatures and prolonged droughts. Thus, higher concentrations of MDA indicate damage on the cell membrane (Fu and Huang, 2001). The present results of an increase in MDA concentration in the leaf lamina under the 250 AGDD defoliation treatments showed that plants had greater cell damage than plants under less frequent defoliations.

An increase in MDA concentration (i.e., increase in membrane peroxidation) might indicate a reduction in the photosynthesis capacity of the plant (Ogweno et al., 2010; Xu and Zhou, 2006), and then a decrease in above and below ground growth. It was reported that soil water restriction increases lipid peroxidation in leaf cells (Fu and Huang, 2001); however, to our knowledge, no study has previously reported an increase in oxidative stress in plants due to frequent defoliation (Table 4.3).

Following defoliation of temperate grasses, WSC are remobilised primarily from the stubble to re-establish leaf growth (Fulkerson and Donaghy, 2001). After a defoliation event, the highest priority for allocation of assimilates is the reestablishment of a photosynthetic apparatus (i.e., leaf regrowth (Donaghy and Fulkerson, 1998)). Similarly, in the present study, under the most infrequent defoliation, Bv plants replenished their WSC reserves and allocated more assimilates towards root growth.

Both the WSC concentration and content of the stubble of Bv plants were higher under low soil water levels, probably as part of a strategy to enhance survival during a shortage of water and to recover production following the water shortage (Volaire et al., 2005). Part of the higher WSC accumulation would also be a result of lower growth during the soil water restriction. However, Volaire et al. (1998) reported that under a drought, growth ceased within 10 days in Lp and *Dactylis glomerata* L., but the amount of WSC accumulation in the stubble during the drought was different between the species and even between cultivars of *D. glomerata.*

Starch is another polysaccharide that functions as an energy reserve substrate, mostly in tropical C4 grasses, legumes and some temperate C3 grasses such as rice (Smith, 1973). Starch content can increase in temperate grasses during stem elongation and seed-head development (Miyake, 2016; Smith, 1973). The amount of starch found in the Bv plants was low, which suggests the main storage compounds for Bv are WSC. However, as with WSC, starch accumulation depended on the defoliation frequency, indicating that it could also play a role in the regrowth immediately after defoliation as a reserve compound in the stubble.

4.6 CONCLUSIONS

The results from the present study indicated that soil water restriction, from 80–85% FC to 20–25% FC, generated an opportunity for Bv to increase its competitiveness relative to Lp by increasing root growth. The expression of this attribute was enhanced under the low defoliation frequency (1000 AGDD treatment), which demonstrates the importance of the defoliation frequency for Bv growth and its competitiveness in a mixture. This water stress-tolerant grass could improve the amount of forage in New Zealand pastures during summer droughts, which are increasing in severity and occurrence in some parts of the country due to climate change. However, it is relevant to test under field conditions, where the plants are better able to explore the soil horizon.

4.7 REFERENCES

Bartholomew, P., & Williams, R. (2006). Effects of exposure to below-freezing temperatures, soil moisture content and nitrogen application on phyllochron in cool-season grasses. *Grass and Forage Science, 61*(2), 146-153.

Bristiel, P., Roumet, C., Violle, C., & Volaire, F. (2018). Coping with drought: root trait variability within the perennial grass *Dactylis glomerata* captures a trade-off between dehydration avoidance and dehydration tolerance. *Plant and Soil, 434*(1), 327-342.

Calvache, I., Balocchi, O., Alonso, M., Keim, J. P., & López, I. F. (2020). Thermal time as a parameter to determine optimal defoliation frequency of perennial ryegrass (*Lolium perenne* L.) and pasture brome (*Bromus valdivianus* Phil.). *Agronomy, 10*(5), 620.

Chapman, D., & Lemaire, G. (1993). Morphogenetic and structural determinants of plant regrowth after defoliation. In *Proceedings of the XVII International Grassland Congress, Palmerston North and Rockhampton, 1993* (pp. 95-104). Sir Publishing.

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought—from genes to the whole plant. *Functional plant biology*, *30*(3), 239-264.

Clement, A., Dalley, D., Chapman D., Edwards G., & Bryant R. (2016). Effect of grazing system on nitrogen partitioning in lactating dairy cows grazing irrigated pastures in Canterbury, New Zealand. In *Proceedings of the New Zealand Society of Animal Production* (Vol. 76, pp. 94-99). New Zealand Society of Animal Production.

Descalzi, C., Balocchi, O., López, I., Kemp, P., & Dörner J. (2018). Different soil structure and water conditions affect the growing response of *Lolium perenne* L. and *Bromus valdivianus* Phil. growing alone or in mixture. *ournal of soil science and plant nutrition*, *18*(3), 617-635.

Diaz, S., Lavorel S., McIntyre S., Falczuk V., Casanoves F., Milchunas D.G., Skarpe C., Rusch G., Sternberg M., Noy-Meir I. (2007). Plant trait responses to grazing–a global synthesis. *Global Change Biology*, *13*(2), 313-341.

Donaghy, D.J., & Fulkerson, W.J. (1998). Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and forage science*, *53*(3), 211-218.

Durand, J.-L., Onillon, B., Schnyder, H., & Rademacher,I. (1995). Drought effects on cellular and spatial parameters of leaf growth in tall fescue. *Journal of experimental botany*, *46*(9), 1147-1155.

Durand, J.-L., Schäufele, R., & Gastal, F. (1999). Grass leaf elongation rate as a function of developmental stage and temperature: morphological analysis and modelling. *Annals of Botany*, *83*(5), 577-588.

Fort, F., Jouany, C., & Cruz, P. (2013). Root and leaf functional trait relations in Poaceae species: implications of differing resource-acquisition strategies. *Journal of Plant Ecology*, *6*(3), 211-219.

Fu, J., & Huang, B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and experimental Botany, 45*(2), 105-114.

Fulkerson, W., & Slack, K. (1995). Leaf number as a criterion for determining defoliation time for *Lolium perenne:* 2. Effect of defoliation frequency and height. *Grass and forage Science*, *50*(1), 16-20.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence — key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian journal of experimental agriculture, 41*(2), 261-275.

Gastal, F., & Lemaire, G. (2015). Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: review of the underlying ecophysiological processes. *Agriculture, 5*(4), 1146-1171.

Gibson, D. J., & Newman, J.A. (2019). Grasslands and climate change: An overview. In D.J. Gibson & J.A. Newman (Eds.), *Grasslands and climate change* (pp. 3–18). Cambridge University Press.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The american naturalist, 111*(982), 1169-1194.

Grime, J. P., Hodgson, J. G., & Hunt, R. (2014). *Comparative plant ecology: a functional approach to common British species* Springer.

Hall, R. (1978). The analysis and significance of competitive and non-competitive interference between species. In J.R. Wilson. (Ed.), *Plant Relations in Pastures* (pp 163–174). CSIRO.

Harris, W., Forde, B., & Hardacre, A. (1981). Temperature and cutting effects on the growth and competitive interaction of ryegrass and paspalum: 1. Dry matter production, tiller numbers, and light interception. *New Zealand Journal of Agricultural Research*, *24*(3-4), 309-320..

Henessy, K., Fitzharris, B., Bates, N., Harvey, S., Howden, M., Hughes, L., Salinger, J., & Warrick, R. (2007). Australia and New Zealand, Climate Change 2007: Impacts, Adaptation and Vulnerability. *Contributions of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta, 207*(4), 604-611.

Hofer, D., Suter, M., Haughey, E., Finn, J. A., Hoekstra, N. J., Buchmann, N., Lüscher A., & Bennett, J. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology*, *53*(4), 1023-1034.

Hooper, D. U., Chapin, F., Ewel, J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J., Lodge, D., Loreau, M., & Naeem, S. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological monographs*, *75*(1), 3-35.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science*, *70*(2), 283-295.

Kemp, D. R., & Culvenor, R. A. (2010). Improving the grazing and drought tolerance of temperate perennial grasses. *New Zealand journal of agricultural research*, *37*(3), 365-378.

Kemp, P. & López, I. (2016). Hill country pastures in the southern North Island of New Zealand: an overview. *NZGA: Research and Practice Series*, *16*, 289-297.

López, I., Balocchi, O., Lailhacar ,P., & Oyarzun, C. (1997). Characterization of the growing sites of six native and naturalized pasture species in the Dominio Humedo of Chile. *Agro Sur, 25*, 62–80.

López, I., Lambert, M., Mackay, A., & Valentine, I. (2003). The influence of topography and pasture management on soil characteristics and herbage accumulation in hill pasture in the North Island of New Zealand. *Plant and Soil*, *255*(2), 421-434.

López, I. F., Kemp, P. D., Dörner, J., Descalzi, C. A., Balocchi, O. A., & García, S. (2013). Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. And *Lolium perenne* L. plants under water restriction. *Journal of Agronomy and Crop Science*, *199*(6), 449-459.

McMaster, G. S., & Wilhelm, W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and forest meteorology, 87*(4), 291-300..

Miyake, H. (2016). Starch accumulation in the bundle sheaths of C3 plants: a possible pre-condition for C4 photosynthesis. *Plant and Cell Physiology, 57*(5), 890-896..

Mommer, L., Van Ruijven, J., De Caluwe, H., Smit-Tiekstra, A. E., Wagemaker, C. A., Joop Ouborg, N., Bögemann, G. M., Van Der Weerden, G. M., Berendse, F., &

De Kroon, H. (2010). Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. *Journal of Ecology, 98*(5), 1117-1127.

Motte, H., Vanneste, S., & Beeckman, T. (2019). Molecular and environmental regulation of root development. *Annual Review of Plant Biology, 70*, 465-488.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of biological chemistry*, *153*(2), 375-380.

Ogweno, J.O., Hu, W. H., Song, X. S., Shi, K., Mao, W. H., Zhou, Y. H., & Yu, J. Q. (2010). Photoinhibition-induced reduction in photosynthesis is alleviated by abscisic acid, cytokinin and brassinosteroid in detached tomato leaves. *Plant growth regulation*, *60*(3), 175-182.

Ordoñez, I., López, I., Kemp, P., Donaghy, D., Herrmann, P., Hernández, F., & Bhatia, S. (2017). Pasture brome (*Bromus valdivianus*) leaf growth physiology: a six-leaf grass species. *Agronomy New Zealand*, *47*, 13-22

Proulx, R., Wirth, C., Voigt, W., Weigelt, A., Roscher, C., Attinger, S., Baade, J., Barnard, R. L., Buchmann, N., & Buscot, F. (2010). Diversity promotes temporal stability across levels of ecosystem organization in experimental grasslands. *PLoS one*, *5*(10), e13382

Ramírez, M., Keim, J., López, I., & Balocchi, O. (2014). Vegetational dynamics of sown pastures with native and naturalized species with and without fertilizer application. *Agro Sur*, *42*(1), 3-14.

Reich, P. B. (2014). The world-wide ‘fast–slow’plant economics spectrum: a traits manifesto. *Journal of Ecology*, *102*(2), 275-301.

Sanderson, M. A. (2010). Stability of production and plant species diversity in managed grasslands: A retrospective study. *Basic and Applied Ecology*, *11*(3), 216-224.

Sanderson, M. A., Skinner, R. H., Barker, D. J., Edwards, G. R., Tracy, B. F., & Wedia, D. A. (2004). Plant species diversity and management of temperate forage and grazing land ecosystem. *Crop science*, *44*(4), 1132-1144.

Smith, D. (1973). The nonstructural carbohydrates. In G. Butler & R. Bailey (Eds.), *Chemistry and biochemistry of herbage* (pp. 105–155). Academic Press.

Team, R.C. (2013). *R: A language and environment for statistical computing*. R-project.

Teughels, H., Nijs, I., Van Hecke, P., & Impens, I. (1995). Competition in a global change environment: the importance of different plant traits for competitive success. *Journal of Biogeography*, 297-305.

Tilman, D. (2001) Functional diversity. *Encyclopedia of biodiversity*, *3*(1), 109-120..

Tow P.G., Lazenby A. (2000). *Competition and succession in pastures.* CABI.

Turner, L. R., Donagh,y D. J., Lane, P. A., & Rawnsley, R. P. (2006a). Effect of defoliation management, based on leaf stage, on perennial ryegrass (Lolium perenne L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 2. Nutritive value. *Grass and forage science*, *61*(2), 175-181.

Turner, L. R., Donaghy, D. J., Lane, P. A., & Rawnsley, R. P. (2006b). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 1. Regrowth, tillering and water-soluble carbohydrate concentration. *Grass and forage science*, *61*(2), 164-174.

Turner, L., Donaghy, D., Lane, P., & Rawnsley, R. (2007). Patterns of leaf and root regrowth, and allocation of water-soluble carbohydrate reserves following defoliation of plants of prairie grass (*Bromus willdenowii* Kunth.). *Grass and Forage Science*, *62*(4), 497-506.

Turner, N. (1986). Adaptation to Water Deficits: a Changing Perspective. *Functional Plant Biology*, *13*(1), 175-190.

Volaire, F., Norton, M. R., Norton, G. M., & Lelievre, F. (2005). Seasonal patterns of growth, dehydrins and water-soluble carbohydrates in genotypes of Dactylis glomerata varying in summer dormancy. *Annals of botany, 95*(6), 981-990.

Volaire, F., Thomas, H., & Lelièvre, F. (1998). Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: I. Growth, death, water relations and solute content in herbage and stubble. *The New Phytologist*, *140*(3), 439-449..

Woodward, S. J., Van Oijen, M., Griffiths, W. M., Beukes, P. C., Chapman, D. F. (2020). Identifying causes of low persistence of perennial ryegrass (*Lolium perenne*) dairy pasture using the Basic Grassland model (BASGRA). *Grass and Forage Science*, *75*(1), 45-63.

Xu, Z., & Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of experimental botany*, *59*(12), 3317-3325.

Xu, Z. Z., & Zhou, G. S. (2006). Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. *Planta, 224*(5), 1080-1090.

Zhao, Y., Li, Z., Zhang, J., Song, H., Liang, Q., Tao, J., Cornelissen, J. H., & Liu, J. (2017). Do shallow soil, low water availability, or their combination increase the competition between grasses with different root systems in karst soil? *Environmental Science & Pollution Research, 24*(11).

CHAPTER 5

Bromus valdivianus Phil. and *Lolium perenne* L. characteristics that influence sheep dietary preference during the different seasons and time of the day

---

Plant characteristics, such as tiller height, leaf and sheath size, and forage nutritive values are determinant factors on the grazing process by ruminants (i.e., grazing time, number of bites). These factors modify the grazing preference for one species over the other. This grazing preference may change throughout seasons due to differences in growth between species. Therefore, the present chapter evaluated sheep grazing preference between *Bromus valdivianus* and *Lolium perenne* during, winter, spring and summer and at two times of the day.

5.1 ABSTRACT

In a climate change scenario, *Bromus valdivianus* Phil. (Bv) is a feasible option to complement *Lolium perenne* L. (Lp) growth in a temperate pasture, as Bv is more drought resistant. However, little is known about animal preference for Bv that might impacts on its production and persistence. Thus, a field study using a randomised complete block design studied sheep ewe's preference between Lp and Bv. During winter, spring, and summer (morning and afternoon) animal grazing behaviour and pasture morphological and chemical attributes were studied. Ewes showed a higher preference for Lp in winter ($p < 0.05$) and summer in the morning ($p < 0.01$), while no differences were found in spring and summer in the afternoon between the species ($p > 0.05$). In winter, Bv had greater ADF and NDF ($p < 0.001$), and lower pasture height ($p < 0.01$) which negatively affected its preference. The lack of differences between the species in spring was due to an increase in ADF concentration in Lp and the pasture heights of both species being close to the optimal to maximise intake rate. In summer, the animals showed a daily preference pattern, selecting Lp in the morning to assure a greater quality and showing no preference during the afternoon to fill the rumen with higher fibre content. The greater sheath weight per tiller in Bv could have made it less desirable, as the decrease in bite rate in the species was likely due to a higher shear strength and lower pasture sward mass per bite which increased foraging cost. These results provided evidence on how Bv characteristics can influence ewe's preference; but more research is needed on how this will affect Lp and Bv growing as a mixture and to develop strategies to manage grazing during the winter.

5.2 INTRODUCTION

Bromus valdivianus Phil. (pasture brome; Bv) mixed with *Lolium perenne* L. (perennial ryegrass; Lp) pasture, is an option for reducing the risk of climate variations such as drought, and increasing pasture persistence (Keim et al., 2015). *Bromus valdivianus* is a perennial grass with a conservative or stress tolerant strategy, as it has a drought resistance strategy that allows it to continue growing during the summer when soil water availability is low (Keim et al., 2015; López et al., 2013). When soil water availability is scarce Bv can harvest water from deeper soil layers compared to Lp (Ordóñez et al., 2018). Conversely, Bv shows a growth diminishment under waterlogging conditions in the soil (Stewart, 1996) (i.e., conditions presented in different regions of New Zealand during winter); and in particular Bv shows good adaptation to free-draining soils (López et al., 1997). In a one-year study in Chile, Bv herbage mass accumulation was similar to *Lolium perenne* L. (Calvache et al., 2020). In addition, it showed higher protein values than Lp, but less energy content; however, both species showed values above 10 MJ/kg DM (Calvache et al., 2020). Although Bv is commercially available in New Zealand (Nichols et al., 2016) it is not commonly sown. Nevertheless, its utilisation under rainfed conditions is likely to increase in the future due to severe climate change scenarios. Currently, however, little is known about ruminant animal's preference for this species.

New Zealand pastures have experienced production and persistence problems due to climate variations, mainly droughts in some parts of the country (Woodward et al., 2020). Soder et al. (2007) documented the importance of pasture species diversity on animal grazing behaviour and how grazing animals alter pasture diversity. However, the influence of pasture characteristics on sheep preference has not been well explored. Animal preference is the discrimination made by animals among pastures or their components, without any environmental or physical restrictions to access to forage (Allen et al., 2011; Hodgson, 1979). Conversely, animal selection or grazing selection is defined as a function of preference for a plant, or a component modified by animal factors, landscape and pasture heterogeneities (Hodgson, 1979).

Diet preference has been shown to change with animal requirements, as Parsons et al. (1994) showed differences in clover intake compared to perennial ryegrass, between lactating and non-lactating sheep. They found non-lactating sheep had a lower preference for clover. Sheep increase their intake of feed rich in fibre (e.g., grass) near sunset in order to avoid grazing at night by maintaining their rumen fill during the night, which resulted in a diurnal pattern in animal preference probably associated with anti-predator behaviour (Edwards et al., 2008). Overall, without any antinutritional components presented in the offered species and with no biochemical differences among species, Villalba and Provenza (1999) stated that the most important factor affecting sheep preference during a whole feeding period was the energy/kg of dry matter. Similarly, Balocchi et al. (2015) showed a negative relationship between sheep preference and neutral detergent fibre (NDF) concentration. Pasture height is another important factor affecting preference and is closely related with intake rate as it presents a positive relationship with the bite mass (Edwards et al., 1995). Szymczak et al. (2020) found that pasture (in a *Festuca arundinacea* Shreb. monoculture) that was 22.3 cm tall maximised intake rate in sheep, as bite mass was greater compared to lower and higher heights.

There is little literature about cattle selection between Lp and Bv, and no research studying sheep preference between these species have been carried out to date. Cattle selection in a diverse pasture (assessed by grazing intensity of tillers) composed mainly of Lp and Bv in southern Chile was assessed by López et al. (2016). In this study, dairy cows selected Bv in autumn and spring over Lp, due to its greater lamina length and tiller height. However, there were no differences during winter and spring, which could be explained by a higher grazing intensity utilised during those seasons (López et al., 2016). Further research is required to explain any apparent animal preference between these pasture species.

The production and persistence of Bv in a mixture could be negatively affected by grazers, as suggested by López et al. (2016), and therefore it is important to assess animal preference for Bv. Seasonal environmental constraints on pasture growth change throughout the year (mainly due to rainfall and temperature interaction), just as there is also forage quality variation among seasons (Keim et al., 2015). Therefore, if two species grow differently across seasons, then the animal may adjust its preference to the seasonal growth of the species (Somasiri et al., 2020). Addressing these behavioural changes through animal preference may contribute to understanding the relative abundance of the species in a diverse pasture. In the present study, it was hypothesised that sheep would prefer Lp during winter, while in summer they would shift preferential grazing to Bv, and that behaviour is related to the pasture conditions (i.e., pasture nutritive quality and herbage mass quantity) in those seasons. Conversely, during spring there is likely to be a lack of preference as both species grow at similar rates with similar qualities due to the transition from vegetative to reproductive growth. The aim of the present study was to evaluate sheep preference between two perennial grass species (but with different seasonal growth) at two times of the day (morning and late afternoon) and across winter, spring, and summer.

5.3 MATERIALS AND METHODS

5.3.1 Experimental site

The study was carried out at Massey University Pasture and Crop Research Unit, on a Manawatu fine sandy loam (Dystric Fluventric Eutrochrept), during winter and spring 2020 and summer 2021, using monoculture pastures of *Lolium perenne* L. cv. Trojan and

Bromus valdivianus Phil. cv. Bareno which were established in October 2018. The total area was 1944 m^2 divided in eight plots of 244 m^2 each (four plots of each pasture species). The pasture management aimed to graze the pastures when they reached a leaf regrowth stage (LS) of 3.5–4.0 for Bv and 2.5–3.0 for Lp (Fulkerson & Donaghy, 2001; García-Favre et al., 2021). Previous research has shown this defoliation interval allows for the two species to recover their reserves of carbohydrates (Fulkerson & Donaghy, 2001; García-Favre et al., 2021) and that these contrasting leaf stages overlap allowing for grazing to occur at the same time on both pasture types (Chapter 6; Ordoñez et al., 2017). The pastures received 50 kg of N fertiliser, as urea fertiliser (46-0-0) on 20 August 2020. At each observation period (see below), the pastures were in the vegetative stage, avoiding reproductive stem elongation in spring.

5.3.2 Experimental design and treatments

The study was set out in a split plot design with four blocks, in which the main plots were the two pastures species (*L. perenne*; Lp and *B. valdivianus*; Bv) and the subplots (split) were the two grazing observational periods (grazing after sunrise; AM and gazing before sunset; PM). These two periods (2.5 hours each) were selected to match the peak daily grazing activities of sheep (Champion et al., 1994; Penning et al., 1991). Each replicate contained two equal sized areas of each pasture species and were further divided into two subplots, equivalent to the splits of 122 m^2 each, one for the AM and the other for the PM. These areas were arranged to enable the animals to have free access to both pastures species at their choice, as no fence was placed between the pastures. In addition, the access gates to each block (at AM and PM) were located in the area where the pastures were adjacent, thus half of the gate was in each pasture.

The sheep regularly grazed the two species, thus avoiding the novelty effect of an unknown species (Parsons et al., 1994; Tamura et al., 2010). In between the observational periods, sheep grazed in a buffer zone with a *L. perenne* based pasture and a water trough. The observations were carried out over four consecutive days during each season: in Day 1 AM and PM the observations were taken in the subplots within block 1; in Day 2 the procedures were repeated in block 2, and so on for blocks 3 and 4. The observations were carried out in 2020 and 2021 in winter (from 27/07/2020 to 30/07/2020), spring (from 20/09/2020 to 23/09/2020) and summer (01/03/2021 to 04/03/2021). Due to seasonal change in the number of daylight hours, the AM and PM observational periods varied among the three seasons, such that: winter observations were made between 7:30 to 9:30 hr and 15:00 to 17:00 hr; spring, 7:00 to 9:00hr and 17:30 to 19:30 hr; summer 7:00 to 9:00 hr and 18:00 to 20:00 hr.

The same group of 10 Romney sheep was utilised on all occasions. In addition, between 5 and 10 extra ewes (non-core ewes) were added to the main flock to achieve a grazing pressure (relationship between animal live weight and forage mass per unit, as defined by Allen et al. (2011)), of ~ 25%, avoiding high grazing pressures that could increase animal competition and hence reduce the ability of the sheep to display preference (Clark & Harris, 1985). The variation in the number of non-core ewes was dependent on the pre-grazing herbage mass in the plots. The ewes were placed in the plots 30 minutes before each observational period began to allow them time to acclimatise to their surroundings.

5.3.3 Animal measurements

The sheep behaviour was assessed following the procedure by Pain et al. (2015). Briefly, a trained person observed and recorded the number of ewes within plots and their activities (i.e., grazing, chewing, not eating, or lying/sitting). The animal behaviour activities were recorded individually for each of the 10 animals. Grazing time was defined as the period when the animal was actively grazing or selecting forage with its head down. Chewing time was defined as when the animal was chewing the recently grazed pasture and the ruminal bolus with its head up. Not eating if the ewe presented the head up with no visible herbage in its mouth and lying if the sheep was sitting or lying on the ground. The observations were made at 5-min intervals, according to Rook and Huckle (1997). Diet preference was calculated as the percentage of time ewes grazed each pasture species, as shown by Penning et al. (1991).

Between the five minutes of grazing time assessments, bite rate was recorded using the “20 bites method” described by Forbes and Hodgson (1985), which counts the time spent by the animal to take 20 bites. It was recorded three times per sheep in each observation period by a trained person. If the time between two consecutive bites was longer than 15 seconds then the measurement was cancelled and restarted (Balocchi et al., 2002).

5.3.4 Pasture measurements

Herbage mass in each block was measured the day before a grazing event. Pasture samples were taken by cutting the pasture contained within a quadrat to ground level. Three quadrats (20 × 50 cm) per pasture species were collected within each block. The samples were oven dried at 70 °C for 48 hr or until they reached constant weight.

Before grazing, twenty random readings of the pasture surface height were recorded from each pasture species in each plot using a sward stick (Rhodes 1981). In addition, lamina and sheath weight per tiller were measured on 10 random tillers per species in each plot, by cutting them to ground level. They were taken to a laboratory, where leaf regrowth stage was determined and lamina and sheath parts of each tiller were separated, dried and weighed following the same procedure described above. To determine the percentage of lamina dry weight per tiller, in each observational period (AM and PM), five laminas per species were cut at the ligule position and immediately taken to the laboratory to be weighed fresh, then dried and reweighed. The dry weight percentage was calculated by dividing the lamina dry weight by the fresh weight.

Two types of samples were collected in each plot at each observation period to assess the nutritive quality of the pastures. One was taken just before sheep were placed into the plots to determine above-ground herbage mass nutritive quality. Thus, one composite sample was made from 10 subsamples per plot, which were cut to ground level using a 10 cm diameter frame and electric scissors. Other composite sample per plot, made from 10 subsamples was obtained using the hand-plucking method in the PM subplot (De Vries, 1995). A hand plucking sample was based on the observations made after ewes grazed in the AM period, with these samples collected to mimic what sheep consumed (Pain et al., 2015).

All the samples were immediately placed in a -20 °C freezer before freeze-drying. The freeze-dried samples were ground through a 1-mm sieve and sent to the Massey University Nutrition Laboratory (Palmerston North, New Zealand) for wet chemical analysis. These samples were analysed for in-vitro dry mater digestibility [DMD (Roughan & Holland, 1977)], crude protein [CP, using Dumas method (Saint-Denis & Goupy, 2004)], neutral detergent fibre [NDF (Mertens, 2002)], acid detergent fibre (ADF) and lignin (Möller, 2009). In addition, to evaluate the herbage quality degree of discrimination in each parameter, the difference between the nutritive quality of the hand-plucking and the above-ground herbage samples was calculated. Thus, D-CP, D-NDF, D-ADF, D-lignin, and D-DMD were obtained (these parameters were used in the multivariate analysis, explained below).

5.3.5 Statistical analysis

The statistical analysis was performed using R Statistic software (Team, 2013). A linear mixed model, with lmer function from the lmerTest package (Kuznetsova et al., 2017), was used. Thus, a one-way analysis of variance (ANOVA) was performed on the measured variables, to test the significance of the main factors: pasture type, observation period (only for behavioural variables) and their interactions. The blocks were assumed to be a random effect and the other factors as fixed effects. Statistically significant differences ($P \leq 0.05$) among least-square means were tested using lsmeans package (Lenth, 2017).

A canonical variate analysis (CVA) was performed with Candisc package. The variables used were those related to morphological traits of the pastures, percentage of grazing during AM and PM, and the difference between total pasture quality and diet selection by animals. More details about CVA analysis can be found in López, et al. (2003).

5.4 RESULTS

5.4.1 Animal behaviour

Time spent grazing (grazing %) showed significant interaction between pasture type and observational period in winter ($p < 0.05$). Ewes spent almost 50% of their time grazing *L. perenne* during the PM, ~30% during AM, and less than 20% grazing *B. valdivianus* during both periods. The rest of the activities did not show any significant interaction with pasture type. Chewing and lying were higher during AM ($p < 0.001$, and $p < 0.05$; respectively). Bite rate was 17% greater in *L. perenne* ($p < 0.05$) than in *B. valdivianus* and it was 27% faster during PM ($p < 0.001$) (Table 5.1).

During spring, pasture type had no effect on grazing time. The sheep displayed a 39.1% greater grazing time at PM compared to AM time ($p < 0.001$). In addition, lying was modified by pasture type ($p < 0.05$) and observational period ($p < 0.01$), with an increased percentage of time lying on *B. valdivianus* pasture and in the AM time. Bite rate also increased 23.5% during PM ($p < 0.001$), and there were no significant differences between pasture types ($p > 0.05$) (Table 5.1).

In the summer, the interaction of pasture type and observational period was significant for grazing time ($p < 0.01$). Thus, ewes spent less time grazing on Bv pasture than on Lp pasture during the AM, but there was no difference between the pastures during the PM. Ewes also spent more time chewing and lying during the AM ($p < 0.01$ and $p < 0.05$, respectively), compared to PM. Bite rate changed according to the pasture type ($p < 0.001$) and observational period ($p < 0.001$). Bite rate increased by 23.7% on Lp, compared to Bv. Further, the sheep showed 15.2% greater bite rate during the PM period (Table 5.1).

Table 5.1. Bite rate and percentage of time allocated to each pasture type (*Lolium perenne* and *Bromus valdivianus*) and to each measured activity (grazing, chewing, lying, and not eating) by ewes during observational periods at morning (AM) and afternoon (PM). The lamina dry weight of pasture types at AM and PM. Results are reported for winter, spring, and summer.

	Grazing (%)	Chewing (%)	Lying (%)	Not eating (%)	Bite rate (bites/min)	Lamina dry weight (%)
			Winter			
Pasture type						
B. valdivianus	19.3 b	3.0	8.5	6.4	32.6 b	19.0 b
L. perenne	38.8 a	4.5	11.1	8.3	38.3 a	19.8 a
Significance	***	ns	ns	ns	*	*
Observational period						
AM	25.8	5.2 a	14.9 a	4.1 b	29.8 b	17.6 b
PM	32.4	2.2 b	4.6 b	10.8 a	41.1 a	21.3 a
Significance	ns	**	*	***	***	***
Pasture type × Observational period						
B. valdivianus × AM	20.7 c	4.5	11.7	3.1	26.5	16.5 c
L. perenne × AM	30.9 b	6.0	18.2	5.0	33.0	18.8 b
B. valdivianus × PM	18.0 c	1.5	5.3	9.8	38.7	21.6 a
L. perenne × PM	46.8 a	3.0	3.9	11.7	43.6	20.9 a
Significance	*	ns	ns	ns	ns	**
			Spring			
Pasture type						
B. valdivianus	32.5	1.1	18.9 a	4.8	34.9	20.7 a
L. perenne	30.3	1.4	8.3 b	2.6	39.4	18.9 b
Significance	ns	ns	*	ns	ns	**
Observation period						
AM	24.0 b	1.7	20.0 a	4.2	32.2 b	18.0 b
PM	38.9 a	0.7	7.2 b	3.2	42.1 a	21.6 a
Significance	***	ns	**	ns	**	***
Pasture type × Observational period						
Significance	ns	ns	ns	ns	ns	ns
			Summer			
Pasture type						
B. valdivianus	28.5 b	3.6	8.9	2.5	47.5 b	25.9
L. perenne	37.1 a	3.8	13.6	2.1	62.3 a	25.2
Significance	**	ns	ns	ns	***	ns
Observational period						
AM	25.1 b	5.3 a	16.7 a	2.9	50.1 b	22.2 b
PM	40.6 a	2.2 b	5.7 b	1.8	59.1 a	28.9 a
Significance	***	**	*	ns	***	***
Pasture type × Observational period						
B. valdivianus × AM	16.5 b	5.0	11.8	2.6	43.7	21.7
L. perenne × AM	33.7 a	5.5	21.7	3.1	58.1	22.8
B. valdivianus × PM	40.6 a	2.2	5.9	2.4	51.2	30.1
L. perenne × PM	40.5 a	2.2	5.4	1.1	66.8	27.6
Significance	**	ns	ns	ns	ns	ns

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

5.4.2 Pasture morphological parameters and quality

The leaf regrowth stage for Bv was 3.6, 2.6 and 2.6 in winter, spring, and summer, respectively. Conversely, Lp showed values of 2.8, 2.4 and 1.6 in winter, spring, and summer, respectively.

Pasture height and herbage mass were higher for Lp, compared to Bv during winter and spring ($p < 0.01$). Whereas no differences were found in summer. Dead matter was 5.1% higher for Lp in winter, while Bv presented higher lamina weight per tiller in winter (2.3 times; $p < 0.001$), spring (2.4 times; $p < 0.01$) and summer (3.7 times; $p < 0.05$). The weight of sheath per tiller was also greater for Bv, compared to Lp in winter (2.6 times; $p < 0.01$) and spring (1.9 times; $p < 0.01$), with no differences in summer ($p > 0.05$). In addition, the lamina/sheath ratio was only significantly different for summer ($p < 0.001$), with greater values for Bv, compared to Lp (Table 5.2).

Table 5.2. Effect of pasture type (*Bromus valdivianus* and *Lolium perenne*) in pasture height, herbage mass, dead matter, lamina and sheath weight per tiller, and lamina/sheath ratio during winter, spring and summer.

Pasture type	Pasture height	Herbage mass	Dead matter	Lamina weight/tiller	Sheath weight/tiller	Lamina/Sheath
				Winter		
B. valdivianus	14.4 b	1031 b	4.2 b	101.5 a	54.6 a	2.0
L. perenne	22.4 a	1893 a	9.3 a	42.8 b	20.3 b	2.8
Significance	**	***	**	***	**	ns
				Spring		
B. valdivianus	17.8 b	1924 b	6.35	140.7 a	123.5 a	1.5
L. perenne	26.0 a	2558 a	6.45	56.7 b	62.3 b	1.2
Significance	**	*	ns	**	**	ns
				Summer		
B. valdivianus	10.3	1431	37.7	75.1 a	38.8	2.2 a
L. perenne	11.8	1678	42.4	20.2 b	27.6	0.9 b
Significance	ns	ns	ns	*	ns	***

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

Herbage quality changed across seasons. Thus, during winter, Bv presented higher concentration of CP ($p < 0.001$), NDF ($p < 0.001$), ADF ($p < 0.001$), and lignin ($p < 0.01$). However, DMD was 3% higher for Lp. During spring, Bv showed more NDF ($p < 0.05$) and lignin ($p < 0.05$), compared to Lp. In summer, Bv showed greater ADF ($p < 0.001$), and the rest of the parameters were similar between pasture types (Table 5.3).

Table 5.3. Effect of pasture type (*Bromus valdivianus* and *Lolium perenne*) in crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and dry matter digestibility (DMD) presented in the above-ground herbage mass (cut to ground level) and in the herbage mass samples obtained by the hand-plucking method (data is shown in brackets) during winter, spring, and summer.

Pasture	CP (%)	NDF (%)	ADF (%)	Lignin (%)	DMD (%)
Winter					
B. valdivianus	18.4 a (22.0 a)	47.6 a (44.0 a)	26.6 a (22.5)	2.6 a (1.9)	68.4 b (71.1 b)
L. perenne	13.9 b (18.5 b)	43.1 b (39.3 b)	23.0 b (21.3)	1.6 b (1.6)	71.4 a (73.3 a)
Significance	*** (*)	*** (*)	*** (ns)	** (ns)	*** (*)
Spring					
B. valdivianus	17.2 (21.5)	51.5 a (44.7 a)	30.3 (26.8 a)	1.9 a (1.9 a)	69.4 (71.8 b)
L. perenne	14.9 (21.0)	47.0 b (39.3 b)	28.6 (23.3 b)	1.6 b (1.3 b)	71.3 (75.0 a)
Significance	ns (ns)	* (**)	ns (**)	* (*)	ns (**)
Summer					
B. valdivianus	11.8 (18.3)	61.2 (49.5)	36.1 a (28.0 a)	3.4 (3.0)	62.8 (68.6)
L. perenne	11.2 (17.0)	58.6 (47.7)	30.1 b (24.4 b)	3.1 (2.7)	64.9 (70.5)
Significance	ns (ns)	ns (ns)	*** (***)	ns (ns)	ns (ns)

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, not significant ($p > 0.05$).

The herbage quality of the pasture selected by ewes (assessed by hand plucking method) was significantly different between pasture types in winter and spring for NDF concentration ($p < 0.05$ and $p < 0.01$; respectively) and DMD ($p < 0.05$ and $p < 0.01$; respectively). Thus, Lp showed less NDF concentration (~11%) and higher DMD than Bv (3% and 4% for winter and spring, respectively). *Bromus valdivianus* had greater ADF concentration (13%) in spring ($p < 0.01$) and summer ($p < 0.001$), compared to Lp. While Bv showed higher lignin concentration (31%, $p < 0.05$) only in spring compared to Lp. *Bromus valdivianus* had 16% more CP concentration than Lp in winter ($p < 0.05$), whereas no differences were found in the rest of the seasons (Table 5.3).

The CVA explained 86.5% of the total differences between the treatments with a significant Wilks' lambda ($p \leq 0.001$); CAN 1 explained 79.9% ($p \leq 0.001$) and CAN 2 explained 13.9% of the total variation ($p \leq 0.001$) (Figure 5.1). Along CAN 1 the treatments were separated by pasture type, such that Bv in spring and winter were positively related with increasing lamina and sheath weight. The negative direction of CAN 1 was associated with Lp in summer with an increasing bite rate, and also with increasing Lp herbage mass in spring. CAN 2 showed a close relationship between Lp in winter and increasing grazing time, either during AM or PM. The increase in the grazing time was related with an increase in D-ADF and D-NDF (i.e., negative values - close to zero). In addition, CAN 2 showed, in its negative direction, a close association between the greater bite rate, D-DMD and dead matter concentration in the pastures (Figure 5.1).

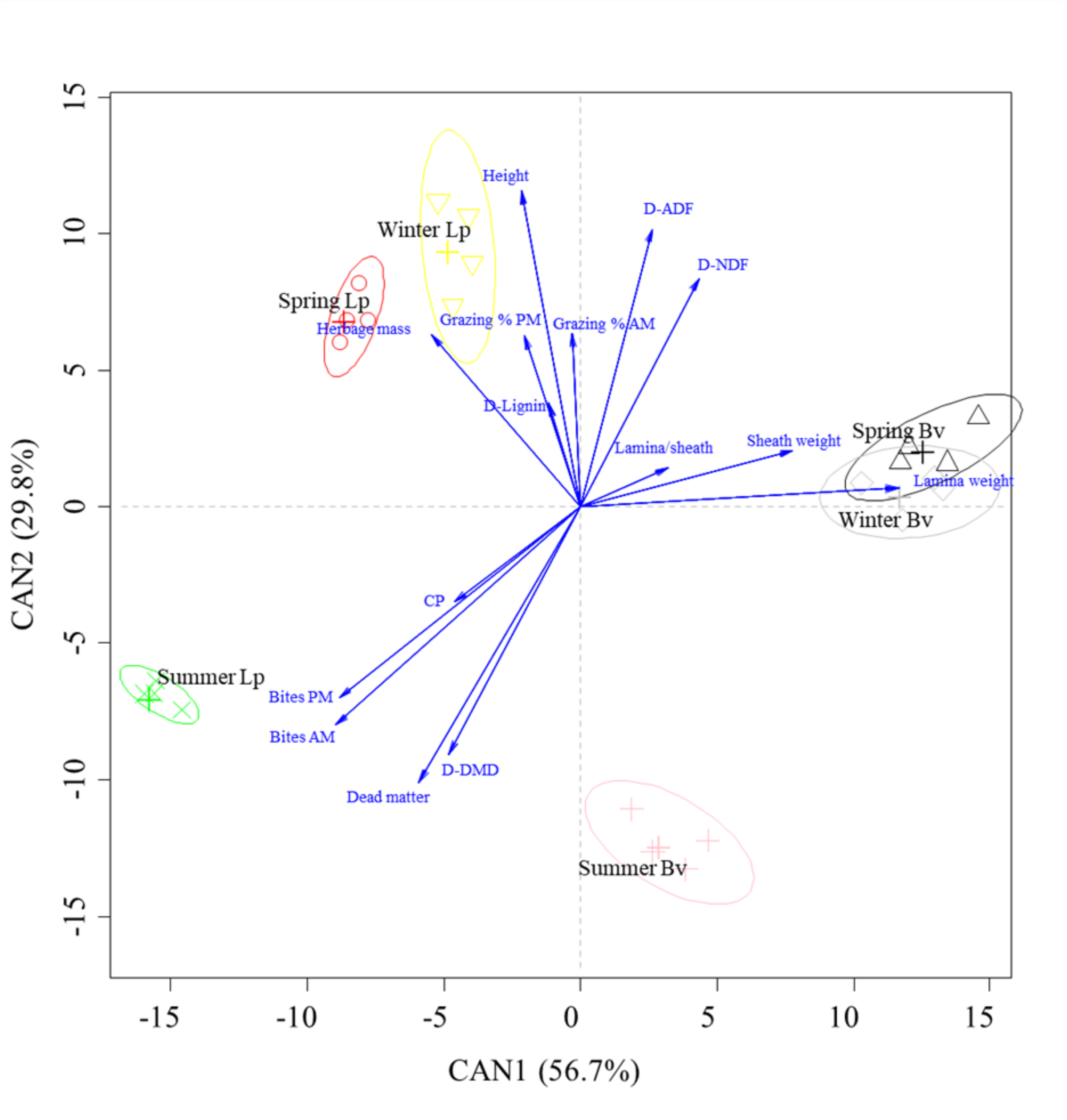


Figure 5.1. Canonical variate analysis for relationship among pasture morphological variables, for the difference between the nutritive quality of the hand-plucking and the above-ground herbage samples in dry matter digestibility (D-DMD), crude protein (C-CP), acid detergent fibre (D-ADF), neutral detergent fibre (D-NDF), and lignin (D-lignin)), and for animal behaviour parameters. Percentage of grazing in the morning; Grazing % AM. Percentage of grazing in the afternoon; Grazing % PM. Number of bites per minute in the morning; Bites AM. Number of bites in the afternoon, Bites PM. *Lolium*

perenne pasture; Lp. *Bromus valdivianus* pasture; Bv. Oval highlighted the 95% confident interval around the means for interaction of pasture type and season.

5.5 DISCUSSION

The partial preference showed in this study by sheep aligns with the theory that animals seek to maintain a healthy rumen (Rutter, 2006). An abundance of desirable pasture species in the animals' diet is paramount to sustain high animal production performance. Therefore, when maximising animal performance, pasture consumption is highly relevant, the partial preferences of the animals must be taken into account to determine which foods they select and avoid (Edwards et al., 2008). The results of the present study highlighted that ewes showed a partial preference for Lp in winter during morning and afternoon periods, and in summer during the morning, without differences in spring (Table 5.1). These results were similar to the partial preference for white clover when was compared with Lp, 70% and 30% of diet, respectively (Parsons et al., 1994).

The preference displayed by sheep for Lp during winter was the result of a combination of pasture parameters. Sheep prefer taller pastures with higher herbage mass (Arnold, 1987; López et al., 2003), as was found in winter for Lp. The energy of the pasture, which is inversely related with the fibre content, is another parameter that can affect animal consumption (Villalba & Provenza, 1999). The 13.5% higher ADF content of Bv during winter could have reduced the animal consumption (Cuchillo-Hilario et al., 2018). Thus, diet preference may depend on the relative energy intake rate of the two alternative species (Newman et al., 1995). The animals probably performed a higher discrimination within Bv components (i.e., leaf blade, sheath) and therefore reached similar values of ADF and lignin in their diet to those obtained from Lp (Table 5.3). This could make Bv less desirable relative to Lp as sheep probably required more energy to perform the discrimination (i.e., increasing walking and handling time) (Garcia et al., 2003; Parsons et al., 1994).

In spring, the animals did not show preference between the species. This can be explained by the pasture height for both species being within the optimal range to reach maximum intake rate (Edwards et al., 1995). Pain et al. (2015) has also observed no preference in spring by sheep when the quality of the pastures (adjacent monocultures of *Plantago lanceolata* L., *Cichorium intybus* L. and *L. perenne*) on offer were similar. Moreover, in spring Bv and Lp presented similarly high levels of ADF and DMD and similar height, herbage mass and dead matter. The lack of difference in herbage quality (except for ADF) and similar pasture height, herbage mass and dead matter could also have an important influence on the lack of animal preference during summer at PM. Therefore, this would explain why sheep did not show any attempt to select a better-quality diet in summer, as they exhibited in winter. In addition, the diurnal pattern in animal preference (shifting preference behaviour from AM to PM) was only shown in summer. This was an indication that in the morning, Lp assured a more rapid intake of nutrients than Bv. Caram et al. (2021), in natural grasslands in Uruguay, observed that cattle showed preference for high quality forage in the morning (CP concentration in this case), while in the afternoon animals increased preference for herbage with higher fibre content. Therefore, adding more quantities of Bv (increasing non digestible fibre intake) to the diet in the afternoon probably was an effective way to favour the rumination process during the night and to keep the ewes alert for predators (Newman, 1995). In addition, one of the most plausible theories for a mixed dietary intake is to maintain a healthy and functional rumen (Edward, 2008; Rutter, 2006).

Sheep are more selective than cattle (Cuchillo-Hilario et al., 2018), and this is likely the main factor that drives differences between the present study and results obtained by

López et al. (2016). In the latter, no differences between species were found; only differences in grazing intensity, being higher for Bv, however that appeared to be driven by tiller height (i.e., Bv tiller height was greater) (López et al., 2016).

The volume of pasture canopy is an important variable that affects intake rate (Tharmaraj et al., 2003). This modifies bite mass and bite rate. In the present experiment, bite rate was always lower in Bv, probably because of a greater time of bite formation (Fonseca et al., 2013). A greater sheath mass and a lower tiller number per plant and per area, a Bv intrinsic characteristic (Descalzi et al., 2018; López et al., 2013), increased the distance of plant aerial organs in the pasture, called divarication, which increased the pasture searching (and choice of bite location) and handling by the animals, decreasing the bite rate in Bv (constrained bite formation) (Fonseca et al., 2013; Prache, 1997). Contrasting with what was found by Illius et al. (1992), the present study showed that the animal bite rate and preference can be more strongly influenced by sheath weight/tiller (and tiller size) than by pasture height. Recently, it was reported that pre-grazing pasture height did not modify bite rate in *F. arundinacea* pasture (pasture height ranging from 14 to 26 cm), while bite mass increased with pasture height up to 22.3 cm, decreasing thereafter (Szymczak et al., 2020). In addition, they showed bite mass, in the grazing process, as the major variable explaining intake rate. Bite mass is mainly modified by bite depth (bite mass depends on bite volume, (Edwards et al., 1995)), which can be depressed by a high amount of sheaths in the pasture due to high tensile resistance of sheath negatively affecting bite formation (Benvenutti et al., 2006). Sheep prefer pasture structures that allow greater speed of ingestion (Black & Kenney, 1984). Therefore, the pasture structure could make Bv less preferred, as ewes would need to spend more energy to harvest a similar bite mass than in Lp.

In a study of sheep preference among different legumes, the ADF concentration explained the major variation in the results, as sheep avoided plants with high ADF content (Thomas et al., 2010). In the present study, the CVA, canonical variate analysis, showed D-ADF was the main driver in the ewe preference in the morning, as indicated by grazing percentage during the AM (i.e., the animals demonstrated a greater discrimination, selecting against ADF content, in Bv than in Lp. [Table 5.3]). In addition, it showed that during summer the animals made a greater effort to increase the DMD in the diet than in the other seasons (i.e., higher D-DMD compared to winter and spring). It reflected a positive association between D-DMD and bite number (CAN 2 in Figure 5.1). This result can be explained according to what was reported by Garcia et al. (2003) that there is a compensatory mechanism (increased searching and walking) by sheep in summer to maximise digestibility of the diet selected instead of reducing the intake rate.

A binary mixture of Lp and Bv could alleviate the divarication that occurs in the Bv monoculture, as Lp could occupy the empty space between tillers of Bv. This may increase the intake rate (Szymczak et al., 2020). Probably, in order to avoid high Lp selection, in winter a high instantaneous stocking rate should be used. However, this would have implications on the grazing behaviour of animals, as according to Teixeira et al. (2017) cows performed less grazing and more aggressive behaviour under a higher instantaneous stocking rate (200 vs 500 cows/ha).

5.6 CONCLUSIONS

The present study provided evidence that ewes show partial preference for Lp in winter and in the morning during summer mainly due to the higher fibre contents of the Bv in that period. Sheath weight/tiller and, thus, tiller size are important pasture structures that can affect the partial preference between Bv and Lp. This study clarifies important pasture characteristics that need to be considered in future studies to maintain a stable mix of Bv and Lp.

5.7 REFERENCES

Allen, V. G., Batello, C., Berretta, E., Hodgson, J., Kothmann, M., Li, X., . . . Peeters, A. (2011). An international terminology for grazing lands and grazing animals. *Grass and Forage Science*, *66*(1), 2-28.

Arnold, G. (1987). Influence of the biomass, botanical composition and sward height of annual pastures on foraging behaviour by sheep. *Journal of Applied Ecology*, 759-772.

Askin, D. C. (1990). Pasture establishment. In R. H. M. Langer (Ed.). *Pastures—Their Ecology and Management* (pp. 132–157). Oxford University Press.

Balocchi, O., Pulido, R., & Fernández, J. (2002). Comportamiento de vacas lecheras en pastoreo con y sin suplementación con concentrado. *Agricultura Técnica*, *62*(1), 87-98.

Benvenutti, M. A., Gordon, I. J., & Poppi, D. P. (2006). The effect of the density and physical properties of grass stems on the foraging behaviour and instantaneous intake rate by cattle grazing an artificial reproductive tropical sward. *Grass and Forage Science*, *61*(3), 272-281.

Black, J., & Kenney, P. (1984). Factors affecting diet selection by sheep. 2. Height and density of pasture. *Australian Journal of Agricultural Research*, *35*(4), 565-578.

Calvache, I., Balocchi, O., Alonso, M., Keim, J. P., & F. López, I. (2020). Thermal time as a parameter to determine optimal defoliation frequency of perennial ryegrass (*Lolium perenne* L.) and pasture brome (*Bromus valdivianus* Phil.). *Agronomy*, *10*(5), 620.

Caram, N., Casalás, F., Soca, P., Anfuso, V., García-Favre, J., Wallau, M., ... & Boggiano, P. (2021). Configuration of daily grazing and searching of growing beef cattle in grassland: observational study. *Animal, 15*(9), 100336.

Champion, R., Rutter, S., Penning, P., & Rook, A. (1994). Temporal variation in grazing behaviour of sheep and the reliability of sampling periods. *Applied Animal Behaviour Science, 42*(2), 99-108.

Clark, D., & Harris, P. (1985). Composition of the diet of sheep grazing swards of differing white clover content and spatial distribution. *New Zealand Journal of Agricultural Research, 28*(2), 233-240.

Cuchillo-Hilario, M., Wrage-Mönnig, N., & Isselstein, J. (2018). Forage selectivity by cattle and sheep co-grazing swards differing in plant species diversity. *Grass and Forage Science, 73*(2), 320-329.

De Vries, M. W. (1995). Estimating forage intake and quality in grazing cattle: a reconsideration of the hand-plucking method. *Rangeland Ecology & Management/Journal of Range Management Archives, 48*(4), 370-375.

Descalzi, C., Balocchi, O., López, I., Kemp, P., & Dörner, J. (2018). Different soil structure and water conditions affect the growing response of *Lolium perenne* L. and *Bromus valdivianus* Phil. growing alone or in mixture. *Journal of soil science and plant nutrition, 18*(3), 617-635.

Edwards, G., Parsons, A., Penning, P., & Newman, J. (1995). Relationship between vegetation state and bite dimensions of sheep grazing contrasting plant species and its implications for intake rate and diet selection. *Grass and Forage Science, 50*(4), 378-388.

Edwards, G. R., Parsons, A. J., & Bryant, R. H. (2008). Manipulating dietary preference to improve animal performance. *Australian Journal of Experimental agriculture, 48*(7), 773-779.

Fonseca, L., Carvalho, P. d. F., Mezzalira, J., Bremm, C., Galli, J., & Gregorini, P. (2013). Effect of sward surface height and level of herbage depletion on bite features of cattle grazing Sorghum bicolor swards. *Journal of animal Science, 91*(9), 4357-4365.

Forbes, T., & Hodgson, J. (1985). Comparative studies of the influence of sward conditions on the ingestive behaviour of cows and sheep. *Grass and forage science, 40(*1), 69-77.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture, 41*, 261-275.

García-Favre, J., López, I. F., Cranston, L. M., Donaghy, D. J., & Kemp, P. D. (2021). The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels. *Agronomy, 11*(2), 300.

Garcia, F., Carrère, P., Soussana, J.-F., & Baumont, R. (2003). The ability of sheep at different stocking rates to maintain the quality and quantity of their diet during the grazing season. *The Journal of Agricultural Science, 140*(1), 113-124.

Hodgson, J. (1979). Nomenclature and definitions in grazing studies. *Grass and Forage Science, 34*(1), 11-17.

Illius, A., Clark, D., & Hodgson, J. (1992). Discrimination and patch choice by sheep grazing grass-clover swards. *Journal of animal ecology*, 183-194.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science, 70*(2), 283-295.

López, I., Balocchi, O., Lailhacar, P., & Oyarzun, C. (1997). Characterization of the growing sites of six native and naturalized pasture species in the Dominio Humedo of Chile. *Agro Sur, 25*, 62-80.

López, I., Hodgson, J., Hedderley, D., Valentine, I., & Lambert, M. (2003). Selective defoliation by sheep according to slope and plant species in the hill country of New Zealand. *Grass and Forage Science, 58*(4), 339-349.

López, I. F., Balocchi, O. A., Alvarez, X., Flores, P., & Latrille, L. (2016). Selectivity of *Bromus valdivianus* Phil., *Lolium perenne* L. and *Agrostis capillaris* L. by grazing dairy cows. *Agro Sur, 44*(3), 53-65.

López, I. F., Kemp, P. D., Dörner, J., Descalzi, C. A., Balocchi, O. A., & García, S. (2013). Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. and *Lolium perenne* L. plants under water restriction. *Journal of Agronomy and Crop Science, 199*(6), 449-459.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC international*, *85*(6), 1217-1240.

Möller, J. (2009). Gravimetric determination of acid detergent fibre and lignin in feed: interlaboratory study. *Journal of AOAC International*, *92*(1), 74-90.

Newman, J., Parsons, A., Thornley, J. H., Penning, P., & Krebs, J. (1995). Optimal diet selection by a generalist grazing herbivore. *Functional ecology*, 255-268.

Nichols, S., Crush, J., Eady, C., Faville, M., Ghamkhar, K., & Woodfield, D. (2016). Future forage plants for hill country systems. *NZGA: Research and Practice Series, 16*, 233-242.

Ordoñez, I., López, I., Kemp, P., Donaghy, D., Herrmann, P., Hernández, F., & Bhatia, S. (2017). Pasture brome (*Bromus valdivianus*) leaf growth physiology: a six-leaf grass species. *Agronomy NZ, 47,* 13-22.

Ordóñez, I., López, I. F., Kemp, P. D., Descalzi, C. A., Horn, R., Zúñiga, F., . . . Dörner, J. (2018). Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. *Soil and Tillage Research, 178,* 55-64.

Pain, S. J., Corkran, J. R., Kenyon, P. R., Morris, S. T., & Kemp, P. D. (2015). The influence of season on lambs' feeding preference for plantain, chicory and red clover. *Animal Production Science, 55*(10), 1241-1249.

Parsons, A., Newman, J., Penning, P., Harvey, A., & Orr, R. (1994). Diet preference of sheep: effects of recent diet, physiological state and species abundance. *Journal of animal ecology,* 465-478.

Parsons, A., Thornley, J. H., Newman, J., & Penning, P. (1994). A mechanistic model of some physical determinants of intake rate and diet selection in a two-species temperate grassland sward. *Functional ecology,* 187-204.

Penning, P., Parsons, A., Orr, R., & Treacher, T. (1991). Intake and behaviour responses by sheep to changes in sward characteristics under continuous stocking. *Grass and Forage Science, 46*(1), 15-28.

Penning, P., Rook, A., & Orr, R. (1991). Patterns of ingestive behaviour of sheep continuously stocked on monocultures of ryegrass or white clover. *Applied Animal Behaviour Science, 31*(3-4), 237-250.

Prache, S. (1997). Intake rate, intake per bite and time per bite of lactating ewes on vegetative and reproductive swards. *Applied Animal Behaviour Science, 52*(1-2), 53-64.

Rook, A., & Huckle, C. (1997). Activity bout criteria for grazing dairy cows. *Applied Animal Behaviour Science, 54*(2-3), 89-96.

Roughan, P. G., & Holland, R. (1977). Predicting in-vivo digestibilities of herbages by exhaustive enzymic hydrolysis of cell walls. *Journal of the Science of Food and Agriculture, 28*(12), 1057-1064.

Rutter, S. M. (2006). Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. *Applied Animal Behaviour Science, 97*(1), 17-35.

Saint-Denis, T., & Goupy, J. (2004). Optimization of a nitrogen analyser based on the Dumas method. *Analytica Chimica Acta, 515*(1), 191-198.

Soder, K. J., Rook, A. J., Sanderson, M. A., & Goslee, S. C. (2007). Interaction of plant species diversity on grazing behavior and performance of livestock grazing temperate region pastures. *Crop Science, 47*(1), 416-425.

Somasiri, S. C., Kenyon, P. R., Morel, P. C. H., Morris, S. T., & Kemp, P. D. (2020). Selection by lambs grazing plantain (*Plantago lanceolata* L.), chicory (*Cichorium intybus* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.) across Seasons. *Animals, 10*(12), 2292.

Stewart, A. (1996). Potential value of some Bromus species of the section Ceratochloa. *New Zealand Journal of Agricultural Research, 39*(4), 611-618.

Szymczak, L. S., de Moraes, A., Sulc, R. M., Monteiro, A. L. G., Lang, C. R., Moraes, R. F., . . . de Faccio Carvalho, P. C. (2020). Tall fescue sward structure affects the grazing process of sheep. *Scientific RepoRtS, 10*(1), 1-10.

Tamura, Y., Yoshida, T., Rikimaru, K., Imanari, M., Fujimura, S., Al-Mamun, M., & Sano, H. (2010). Bioactivity and practical use of plantain (*Plantago lanceolata*). *Proceedings of the New Zealand Grassland Association, 72*, 257-262.

Team, R. C. (2013). R: A language and environment for statistical computing.

Teixeira, D. L., Machado Filho, L. C. P., Hötzel, M. J., & Enríquez-Hidalgo, D. (2017). Effects of instantaneous stocking rate, paddock shape and fence with electric shock on dairy cows' behaviour. *Livestock Science, 198*, 170-173.

Tharmaraj, J., Wales, W., Chapman, D., & Egan, A. (2003). Defoliation pattern, foraging behaviour and diet selection by lactating dairy cows in response to sward height and herbage allowance of a ryegrass-dominated pasture. *Grass and Forage Science, 58*(3), 225-238.

Thomas, D., Milton, J., Revell, C., Ewing, M., Dynes, R., Murray, K., & Lindsay, D. (2010). Preference of sheep among annual legumes is more closely related to plant nutritive characteristics as plants mature. *Animal Production Science, 50*(2), 114-123.

Villalba, J. J., & Provenza, F. D. (1999). Effects of food structure and nutritional quality and animal nutritional state on intake behaviour and food preferences of sheep. *Applied Animal Behaviour Science, 63*(2), 145-163.

Woodward, S. J. R., Van Oijen, M., Griffiths, W. M., Beukes, P. C., & Chapman, D. F. (2020). Identifying causes of low persistence of perennial ryegrass (Lolium perenne) dairy pasture using the Basic Grassland model (BASGRA). *Grass and Forage Science*, *75*(1), 45-63.

CHAPTER 6

A simple binary mixture of two perennial grass species enhanced pasture production under a leaf regrowth stage defoliation criterion in New Zealand rainfed conditions

Plant species richness in pastures enhances ecosystem sustainability. It has been assessed the effect of increasing the number of species on pasture production and growth stability along the year. However, little is known about these parameters when mixing two perennial grasses that differ in plant structure (e.g., tiller size, root depth), and in drought and waterlogging resistance. In addition, how species interact under different defoliation frequencies based on species optimal leaf regrowth stage has received little attention in the literature. The present chapter aimed to evaluate the growth response of *Bromus valdivianus* and *Lolium perenne* mix under two defoliation frequencies (i.e., based on species optimal leaf regrowth stage) in the field over two years.

6.1 ABSTRACT

Lolium perenne L. is the major pasture species sown in New Zealand, but climate change increases the likelihood of drought in some regions during summer and consequent lower growth rates. *Bromus valdivianus* Phil. is a more water stress-tolerant species but its competitiveness in a diverse pasture may depend on the defoliation management and soil moisture levels. The performance (production, persistence and feed quality) of both species under different defoliation regimens was compared when the species were mixed and in monoculture. The treatments, *Bromus valdivianus* and *Lolium perenne* monocultures and the 50/50 mixture, grazed at two defoliation frequencies (i.e., based on *B. valdivianus* and *L. perenne* optimal leaf regrowth stage [LS], respectively: Bv LS and Lp LS) were applied in a completely randomised block design with three blocks. Herbage mass production was 15% higher in the mixture, mainly due to an increase in production in spring and summer. In spring there was complementarity growth between the species, while during summer/early autumn the increase was due to a higher participation of *B. valdivianus* in the mixture. Root biomass at depth (31 – 70 cm) was 38% greater for *B. valdivianus* monoculture and the mixture compared with *L. perenne* monoculture, may have contributed to the better production of the mixture during and following dry periods. In addition, the maintenance of the *B. valdivianus* tiller population during dry periods demonstrated its better fitness to low soil water availabilities than *L. perenne.* Although herbage mass was not modified by defoliation frequency (species were defoliated within their optimal LS range), forage quality of *B. valdivianus* showed values at low defoliation frequency that could compromise animal production during spring. However, there was an increase in root biomass when defoliation was based on Bv LS, which suggested a flexible grazing management across seasons to increase niche utilisation between species in the mixture. The defoliation frequency of the mixture could be based on *L. perenne* LS

during spring and winter, and slow down (following *B. valdivianus* criteria) during summer and autumn.

6.2 INTRODUCTION

Soil moisture deficit is a major threat to pastoral-based agriculture systems (Narasimhan & Srinivasan, 2005), and it is expected to increase in the future during the summer period in New Zealand in some part of the country as a result of climate change (Henessy et al., 2007). Sustainability improvements, low-input farming systems, and water and soil conservation schemes by governments are encouraging farmers around the world to utilise production strategies that allow them to reach those aims. Research has focused on the positive effect of increased grassland richness on ecosystem processes (Tilman et al., 1997). However, a consequence of the increased use of diverse pastures means that their agricultural management can be more complex (Sanderson et al., 2007). In a mixed pasture, seasonal variation in botanical composition, on a dry matter (DM) basis, is part of the typical asynchrony of species growth (Husse et al., 2016). The negative effects of competition for light between species can be diminished by defoliation events that occur at 95% of light interception (Borer et al., 2014). Thus, species succession in a diverse pasture is related to the defoliation regime applied, mainly imposed as the frequency and intensity of grazing (Duru et al., 2014), as well as the animal's diet selection (Grace et al., 2019), among other factors.

Increasing the number of species from differing functional groups (e.g., grass, legumes, forbs) in a pasture results in increased accumulation of herbage mass compared to monocultures of the same species (Nyfeler et al., 2009; Tilman, 2001; Tilman et al., 1997). This increase is sustained by a maximisation of niche complementarity (Nyfeler et al., 2009), asynchrony of shoot growth across seasons (Husse et al., 2016), and synergistic interaction among the species (e.g., utilisation of biologically-fixed nitrogen (N) by non-N fixing species; (Hofer et al., 2016)). While mixtures achieve higher herbage production than monocultures under a wide range of environmental conditions, this response can be intensified under drought conditions (Hofer et al., 2016). Furthermore, under a drought, the effect of defoliation management intensification (i.e. increase in defoliation frequency) was more significant than species richness, as frequent defoliations impaired resistance of grasslands to drought regardless of the number of species present in the pasture (Vogel et al., 2012). Intensively managed pastures, under a regime of high defoliation frequency, are important to sustain the nutrition of ruminants in intensive animal production systems (O'Mara, 2012). Enhancement of the ecosystem multifunctionality of intensively managed pastures, assessed through the response of forage production, N cycling, and forage quality across ecosystems, can be achieved by a low increment in functional species number (up to four species) (Suter et al., 2021). These intensively managed pastures with a low number of species differ from studies of extensively managed pastures using up to 60 species (e.g. Vogel et al. (2012), Roscher et al. (2011)).

In New Zealand pastoral systems, a simple binary sowing mix (two species pasture) composed of *Lolium perenne* L. (Lp) and *Trifolium repens* L. (with a composition ratio of 80-90% and 10-20% *L. perenne* and *T. repens*, respectively) predominates (Pembleton et al., 2015). The intensification of the dry periods during has negatively affected the production and persistence of New Zealand’s rainfed pastures summer in some parts of the country (e.g., eastern regions) (Lee et al., 2017; Woodward et al., 2020). *Lolium perenne* is an exploitative species (i.e. high resource utilisation) or a competitor using the competitor, stress-tolerator and ruderal (CSR) model (Grime et al., 2014), with high above-ground biomass growth under moderate and high water level conditions (Lee et al., 2012). *Lolium perenne* maintains around 75% of its root system in the top 30 cm of soil, and consequently its herbage production significantly diminishes under severe soil moisture deficit events (Hoekstra et al., 2014; Hofer et al., 2016). Thus, the inclusion of drought-tolerant species such as *Plantago lanceolata* L. or *Cichorium intybus* L. into New Zealand pastoral systems was shown to enhance herbage production during the summer period (Pembleton et al., 2015).

Deeper-rooted forage species and/or forage species with a greater proportion of roots at depth, show an increase in drought resistance (defined as “the degree to which a variable is changed, following a perturbation” (Pimm, 1984)), as a dehydration avoidance strategy due to the ability to uptake water from deep soil layers (Volaire, 2018). *Bromus valdivianus* Phil. (Bv) is a perennial grass species well adapted to high fertility and free-drained soil (López et al., 1997). In a study in Chile, its annual herbage mass production and quality were similar to Lp (Calvache et al., 2020) but Ordóñez et al. (2018) showed a higher water uptake from deeper soil layers during summer, and Keim *et al.* (2015) recorded greater growth rate during summer droughts. According to López et al. (2013), Bv has a different growth strategy compared to Lp in how it responds to environmental stress, with greater tiller size (~ 2.3 times) but fewer tillers per plant (~ 2.2 times; (Descalzi et al., 2018; López et al., 2013)). Recently, Suter et al. (2021) showed that, even with a low proportion of legumes in a pasture mixture, the inclusion of deep-rooted species (*Dactylis glomerata* L. in this case) can favour higher pasture production and temporal stability of production. Although Lp and Bv could complement each other and enhance pasture functionality, this may depend on the defoliation management pursued.

Globally, livestock grazing is the largest anthropogenetic land use (Diaz et al., 2007), and defoliation management decisions vary depending on the indicator used to define the timing of defoliation (Fulkerson & Donaghy, 2001). Pasture defoliation frequency based on leaf regrowth stage (LS) assures a replenishment of water-soluble carbohydrates (WSC) in the plant and a fast regrowth after defoliation until the photosynthetic tissue is capable of net carbon assimilation (Fulkerson & Donaghy, 2001). Hence, if grazing occurs when too few leaves/tiller have regrown since the previous grazing (e.g. < 2 for *L. perenne*), the capacity of the plant to survive in stressful conditions (low soil water content, extreme temperatures) is reduced, as the level of WSC 'reserves' will be low (Donaghy et al., 2008). This effect, along with the senescence of the first fully-expanded leaf as regrowth progresses (coinciding with a decline in pasture quality) determine an optimum range of LS recommend for grazing (Fulkerson & Donaghy, 2001). It was shown that the optimum LS for production and persistence of Lp lies between 2.0–3.0 leaves/tiller (Fulkerson & Donaghy, 2001), while for Bv it is between 3.5–4.0 leaves/tiller (García-Favre et al., 2021). In a mixture, to achieve a good pasture production and persistence, both species should be defoliated close to their optimum LS. However, the degree of overlap of LS of Lp and Bv in a mixture has not yet been assessed. To date, only one study has compared defoliation management of a mixture based on the LS of species and this was in a *D. glomerata* L. and *Bromus willdenowii* Kunth mixture (Gatti et al., 2017). It is difficult to optimally graze all species simultaneously in a mixed pasture, as any chosen grazing management will be suboptimal for some species. Therefore, under New Zealand rainfed conditions, the aim was to evaluate whether a simple mixture of two perennial grasses, compared with their monocultures, could enhance herbage production and quality by asynchrony in growth between the two species across seasons. Specific evaluations were: (i) the species dynamics in the mixture when defoliated based on the optimum LS of each species, (ii) the herbage mass production and quality of pastures over two years in relation to defoliation frequency, (iii) the effect of defoliation frequency and the interaction with pasture type on species persistence through tiller dynamics, and root biomass accumulation at the end of the study.

6.3 MATERIALS AND METHOD

6.3.1 Site and treatments

The study was undertaken at Massey University Pasture and Crop Research Unit (40°22'55.8"S 175°36'23.8"E) over a period of two years (March 2019 to March 2021), on a Manawatu fine sandy loam (Dystric Fluventric Eutrochrept), classified as a weathered fluvial recent (Hewitt, 2010). Previously, bulk densities of 1.1 and 1.2 mg m^{-3} in the topsoil, ~ 1.3 mg m^{-3} over the 15 cm soil depth, and a low soil water storage capacity (~ 87 mm in 100 cm depth) were reported (Senarath, 2003). The chemical conditions of soil samples collected to a depth of 7.5 cm before sowing were analysed. The results were: pH 5.0 [1:2 (soil:water)], 39 mg L^{-1} Olsen phosphorus (P), 0.31 me 100 g^{-1} potassium (K), 6.5 me 100 g^{-1} calcium, 1.24 me 100 g^{-1} magnesium, 15 me 100 g^{-1} cation exchange capacity and 9.5 mg kg^{-1} sulphate sulphur (S). During sowing, the soil was fertilised with 37 kg/ha N, 25 kg/ha P, 25 kg/ha K and 18.5 kg/ha S.

In October 2018, after ploughing and harrowing a 0.26 ha site, the seedbed was prepared, and three different types of pastures were sown in plots of 324 m^2: (i) a monoculture of Lp cv Trojan (Lp), (ii) a monoculture of Bv cv Bareno (Bv), (iii) a mixture of both species (Mx). The seeding rate was 25 kg/ha for Lp, 45 kg/ha for Bv, and 16 and 30 kg/ha in the mixture for Lp and Bv, respectively. Bareno is the only commercial cultivar available on the New Zealand market, and Trojan was selected as it is a high-yielding cultivar under conditions of low soil water availability (Kerr et al., 2012) and at the time of the study, had a 5-star rating under the Forage Value Index for the lower North Island (https://www.dairynz.co.nz/feed/pasture/pasture-renewal/select-pasture-species/cultivar-selector-tool/?region=2&forage%20type=1).

After 6 months of pasture establishment, in which three light grazings were carried out to stimulate tillering and root development, two defoliation frequency treatments were imposed. The defoliation frequencies were based on the optimal LS window of Lp (2.5 – 3.0 LS) and Bv (3.5 – 4.0 LS). Thus, a combination of two defoliation frequencies and three pasture types (2 x 3 factorial arrangement; 6 treatments) were applied in a randomised complete block design with three replicates. The three pastures managed under the Lp LS frequency were grazed whenever the Lp reached its optimal LS, while the other three pastures were grazed whenever the Bv reached its optimal LS. Therefore, the two defoliation treatments were applied at different times, resulting in 18 and 15 grazing sessions in the two years of evaluation for Lp LS and Bv LS defoliation frequency treatments, respectively. The average accumulated growing degree (AGDD) between two successive grazing over the two year was 350 AGDD and 440 AGDD for Lp LS and Bv LS defoliation frequency treatments, respectively.

The pastures were grazed with ewe lambs for no more than three days per grazing event, with the aim of obtaining a post-grazing residual of 5 cm. The stocking rate ranged between six and seven lambs per plot, equivalent to 200–233 lambs per hectare at grazing and was adjusted between grazing events and plots, depending on the time of year and predicted herbage growth, to ensure the post-grazing residual target was met. This grazing intensity optimises grass growth and persistence (Fulkerson et al., 1994). In addition to the fertilisation at sowing, the pastures were fertilised with 50 kg/ha of N (in the form of urea [46-0-0]) in early spring (2019 and 2020).

The climate at the site is humid temperate. Monthly rainfall, mean temperature and potential evapotranspiration are shown in Figure 6.1 (data source: NIWA/AgResearch Weather Station, Agent No. 21963, Palmerston North, 2021, located ~500 m from the field site). The Penman-Monteith equation was used to calculate potential evapotranspiration, which was compared with monthly rainfall to determine periods of water shortage for optimal pasture growth (Cai et al., 2007).

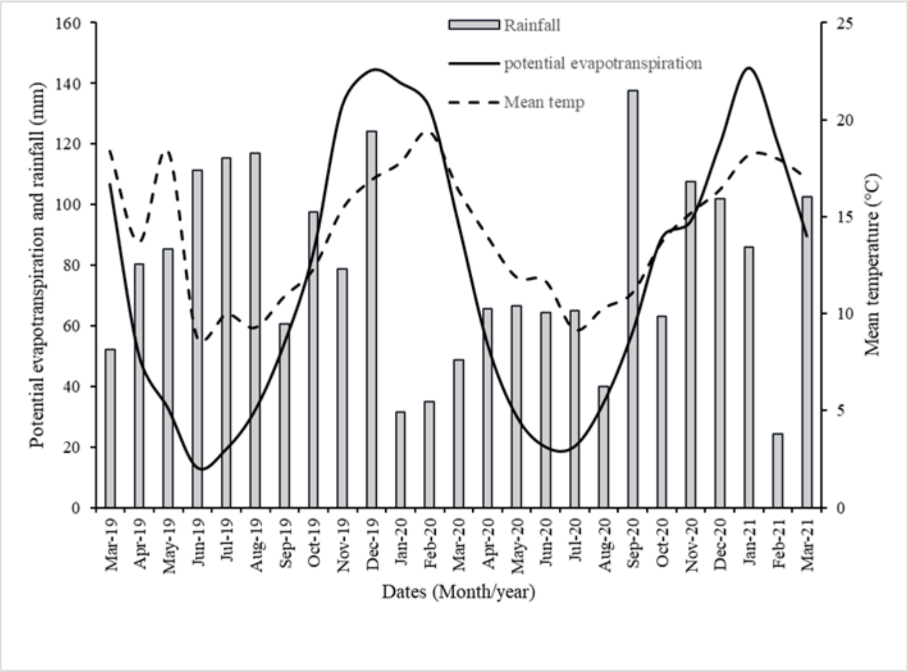


Figure 6.1. Average temperatures during the experimental period (March 2019 to March 2021). Bars indicate rainfall; solid line indicates potential evapotranspiration (using the Penman-Monteith equation) and dashed line indicates mean temperature.

6.3.2 Herbage mass production, botanical composition, and root biomass

Pasture measurements were undertaken when the LS of each monoculture reached its optimal range. Thus, to closely follow the LS development of pastures, the LS was measured in nine tillers per plot every 15 days. At a measurement time, if one of the species was close to its optimum LS, the measurement was repeated before completing the 15 days period, according to how close the target species was to reach its desired LS. In the case of Lp, which grows one leaf at a time, the residual leaf from the prior defoliation was not counted and, thereafter, any new fully expanded leaf (visible ligule) was counted as one, and subsequent leaves recorded to the nearest 0.25 increment (i.e., 0.25, 0.5, 0.75, 1.0). The LS values of each leaf were then added. For Bv, the LS assessment was similar, but considering that for each Bv individual tiller three leaves grow simultaneously, this means that for each tiller, leaves were present at all stages (i.e., 0.25, 0.5, 0.75, 1.0). In addition, the second residual leaf was counted (second leaf from bottom to top, with values of 0.5 or 0.75 according to its length), to capture its continued growth after a grazing event. Therefore, Bv tillers at 3.5 – 4.0 LS had at least 5 leaves, one residual from the previous grazing cycle, at least one or two fully expanded and three growing (García-Favre et al., 2021).

Herbage mass production was recorded as the difference between pre-grazing herbage mass at grazing event ‘n’ and the post-grazing residual at ‘n-1’ (Campbell, 1966). In the pre- and post-grazing, the herbage mass was measured cutting three quadrats (20 × 50 cm) per plot to ground level using an electric manual shearing handpiece. The samples were dried for 72 h in a forced-air oven and weighed. In addition, at pre-grazing the photosynthetically active radiation interception (PAR, 400 – 700 nm) by the canopy was measured with a portable single channel fibre optic probe (PAR Quantum Fibre Optic System, Skye, United Kingdom). This was performed around midday, taking 20 determinations per plot. At each determination, one measurement above the canopy (full light) and immediately below the canopy at ground level were made. Thus, the light intercepted by the canopy was calculated as the ratio between these two readings.

The botanical composition of the pastures was assessed every second grazing session (i.e., at least once per season). For each sample of herbage mass, a subsample (approximately 100 g) was obtained, and the pasture components were manually sorted into ‘weed’, ‘clover’, ‘dead matter’, and depending on the pasture type, into ‘Lp’, ‘Bv’. The components were dried for 72 h in a forced-air oven and weighed.

At the end of the study, root biomass was assessed (15/03/2021 and 24/03/2021 for Bv LS and Lp LS defoliation frequency, respectively). Three soil and root samples were taken per plot using a hydraulic auger of 7 cm diameter. Root samples were collected to a depth of 70 cm, and each core was separated into three depths with a knife: 0 – 10 cm, 11 – 30 cm, and 31 – 70 cm, and placed in separate plastic bags. These layers were selected based on previous knowledge of Lp and Bv root patterns (Deru et al., 2014; Ordóñez, 2020). Each sample was then soaked in water for at least two hours and washed through a 1 mm sieve under running water to wash the roots free from soil. After rinsing the roots several times, the samples were dried for 72 h in a forced-air oven at 60 °C.

6.3.3 Determination of herbage nutritive value

From winter 2018 to autumn 2020, herbage nutritive value of the three pasture types was assessed once per season from the pre-grazing herbage mass. At least 20 subsamples per plot of approximately 10 cm diameter were cut to a residual height of 5 cm. Samples were immediately dried at 60 °C for 72 h in a forced-air oven and ground to pass through a 1.0-mm screen. Then, the samples were sent to the Massey University Nutrition Laboratory (Palmerston North, New Zealand), and crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and organic matter digestibility (OMD) were estimated by near-infrared reflectance spectrometry (NIRS) (Corson et al., 1999). The calibration of the NIRS for each analysis was previously performed by the laboratory after scanning samples of finely-ground pastures in the range of 400 to 2500 nm. The results from the NIRS calibration compared to the wet chemistry analysis had a coefficient of determination (r^2) of 0.90.

6.3.4 Tiller counting

Pasture tiller dynamics were assessed during the two year study following the method described by Ordóñez (2020). Briefly, the number of live tillers per grass species was assessed using a 10 cm diameter (78.5 cm^2) steel ring that had three equidistant welded metallic 1 cm long legs. Each time a determination was performed, the rings were placed in three different permanently fixed positions within each plot. The positions were marked with three galvanised flat-head nails, with dimensions of 150 x 0.6 mm, inserted into the ground. A cleft was made in the head of the nails so that each leg of the ring fit perfectly. Each position within the plots was a subsample (totalling 56 ring positions), and after recording the number of tillers per grass species, the ring was removed to continue to the next position. The nails were found using a metal detector (F22 Metal Detector, Fisher Research Labs, USA). Therefore, at each recording event, the same tiller population was evaluated, which allowed the change in tiller population over time to be captured (i.e., the number at each recording time was the result of tiller death and birth). The recording procedure was performed on all treatments every ~ 20 days, regardless of the regrowth stage. The nails were set up during summer 2019 (establishment stage) at locations with similar pasture conditions (botanical compositions and height), and the first measurement was made on 3 May 2019, while the last measurement was made on 9 March 2021.

6.3.5 Statistical analysis

The statistical analysis was performed using R Statistic software (Team, 2013). A linear mixed model, using the lmer function, from the lmerTest package (Kuznetsova et al., 2017), was used. Thus, a one-way analysis of variance (ANOVA) was performed on the measured variables (herbage mass production, CP, NDF, ADF and OMD), to test the significance of the main factors: pasture type, defoliation frequency, year, season, and their interactions. The blocks were assumed as a random effect and main factors as a fixed effect. The effect of season was nested within year and used in the model as a time-repeated factor (Keim et al., 2015; Pontes et al., 2007). Statistically significant differences ($P \leq 0.05$) among least-square means were tested using lsmeans package (Lenth, 2017), and Tukey test. Statistically significant main factors or any of their interactions that presented a proportion of explained variation less or equal to 0.01 were not considered for the analysis of means (Pontes et al., 2007).

The same procedure was used for root biomass and accumulated herbage mass per year, but without the effect of season and year. An ANOVA was performed for each year and for each soil layer in the case of root biomass. The change in the botanical composition and tiller dynamic of pastures were analysed by standard error of means and graphical representation across dates.

6.4 RESULTS

Overall, the pastures were defoliated at the target LS for Lp (2.5 – 3.0), and Bv (3.5 – 4.0). The mean LS for Lp was 2.7 and 3.1 leaves/tiller for Lp LS and Bv LS, respectively, and the mean LS for Bv was 3.2 and 3.5 leaves/tiller at Lp LS and Bv LS defoliation frequency, respectively. Therefore, there was a difference of 0.4 − 0.5 LS between Lp and Bv. Closely related with the development of leaves, the pre-grazing light interception was 95%, or slightly higher, in all the pastures and seasons, but not during summer (i.e., Lp achieved 90%, Bv 92% and Mx 93%), and during winter for Bv under Lp LS defoliation frequency (90%) (see Appendix 1 for more detailed information).

6.4.1 Total accumulated herbage mass per year, herbage mass production per season and root biomass

For herbage mass production, season as a main factor significantly interacted with pasture type and defoliation frequency ($p \leq 0.001$), explaining 3 and 11% of the total variation, respectively. In addition, pasture type, year and season within year were significant ($p \leq 0.001$), with season showing the highest explained variation (Table 6.1). The herbage mass production of Mx showed the highest values in most of the seasons during the two years, except in the winter of the second year, where Lp achieved the highest production. In spring, Mx produced an average of 1065 kg DM/ha more than Lp and Bv as single-species pastures, while in the second summer that difference reduced to 560 kg DM/ha (Figure 6.2).

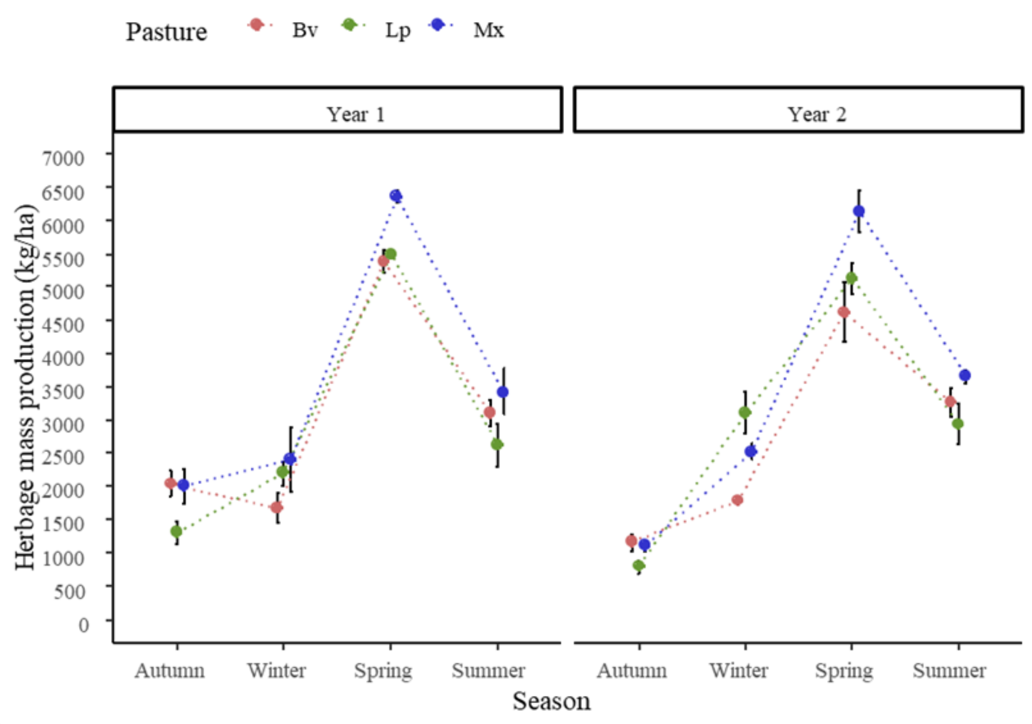


Figure 6.2. Seasonal herbage mass production of *Bromus valdivianus* monoculture (bv), *Lolium perenne* monoculture (lp) and mixture of both species (mx) in different seasons for the first and second year of evaluation. The standard errors of the mean are shown in vertical bars.

Table 6.1. Statistical significance of F ratio values of difference and proportion of explained variation (V) from the analysis of variance for herbage mass production, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and organic matter digestibility (OMD).

	Herbage mass		CP		NDF		ADF		OMD	
	V	F value	V	F value	V	F value	V	F value	V	F value
Pasture	0.02	16.19***	0.29	117.86***	0.07	20.15***	0.10	43.89***	0.29	112.01***
Defoliation frequency	<0.01	0.03NS	0.09	74.59***	0.03	18.08***	0.03	22.59***	0.04	28.67***
Year	0.01	18.27***	0.00	0.00NS	0.02	12.68***	0.03	22.15***	0.01	10.71**
Season(Year)	0.81	199.33***	0.37	60.79***	0.63	73.39***	0.71	121.97***	0.46	70.27***
Pasture×Defoliation frequency	<0.01	1.02NS	0.03	12.74***	0.05	14.14***	0.02	8.49***	0.03	10.55***
Pasture×Year	0.00	0.47NS	0.00	1.03NS	0.00	0.10NS	0.00	0.69NS	0.00	1.25NS
Defoliation×Year	0.00	0.04NS	0.01	12.27***	0.01	5.31*	0.00	3.79NS	0.01	4.68*
Pasture×Defoliation×Year	0.00	0.36NS	0.00	0.35NS	0.00	0.12NS	0.00	0.15NS	0.00	0.12NS
Pasture×Season(year)	0.03	3.75***	0.02	1.96*	0.04	2.17*	0.03	2.99**	0.06	4.31***
Defoliation×Season(year)	0.11	27.57***	0.15	24.25***	0.11	13.34***	0.05	8.51***	0.06	8.60***
Pasture×Defoliation*Season(year)	0.01	0.71NS	0.03	2.44*	0.04	2.64**	0.03	2.68**	0.05	3.57***

Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS, not significant ($p > 0.05$).

Regarding defoliation frequency across seasons within year, the Lp LS defoliation frequency resulted in greater production in summer and autumn of both years and in the first winter compared to Bv LS defoliation frequency (i.e., an average of 580 kg DM/ha more for summer and autumn under high defoliation frequency). However, the opposite occurred in spring of both years and in the second winter, with a higher mean production of 1320 kg DM/ha for the Bv LS compared to the Lp LS defoliation frequency (Figure 6.3).

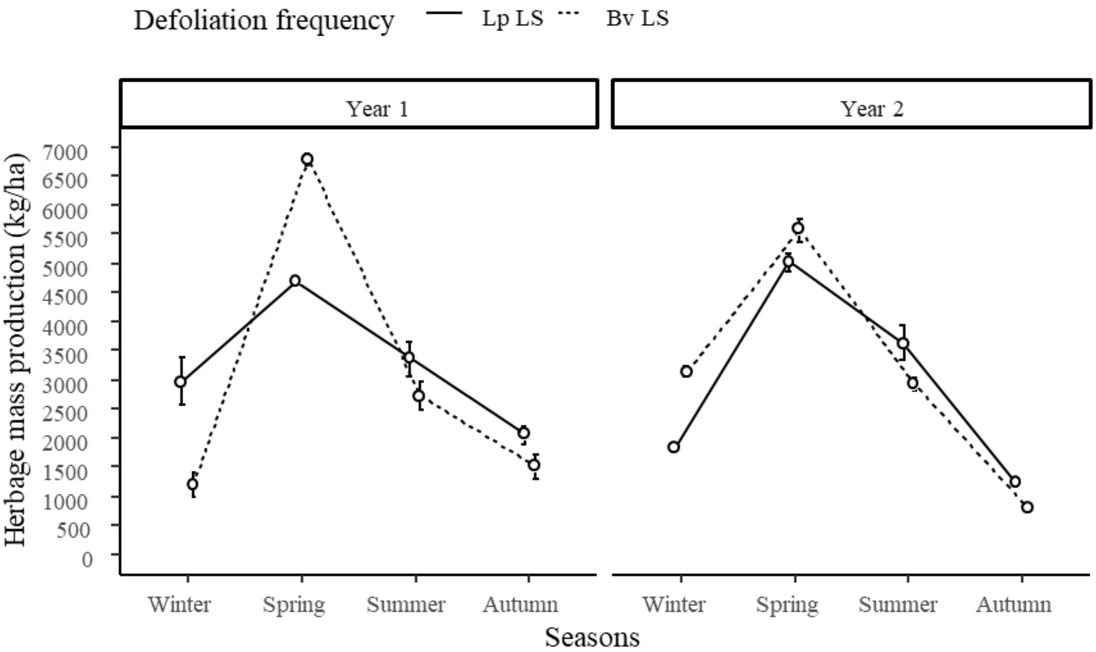


Figure 6.3. Seasonal herbage mass production of the pastures under Lp LS (solid line; based on optimal *L. perenne* leaf regrowth stage) and Bv LS (dashed line; based on *B. valdivianus* optimal leaf regrowth stage) defoliation frequency in different seasons for the first and second year of evaluation. The standard errors of the mean are shown in vertical bars.

Accumulated herbage mass per year was 16.2 and 14.7% greater in Mx for the first and the second year, respectively, compared to the monocultures ($p \leq 0.001$). There were no significant differences between the means of Lp and Bv, for either defoliation frequency, or for any of the interactions between main factors (Table 6.2). In relation to root biomass, evaluated at the end of the study, no differences were found for the first layer (0 – 10 cm), while in the second layer (11 – 30 cm) root biomass increased by 41.5% in Bv under the Bv LS defoliation frequency ($p \leq 0.05$). In the third layer (31 – 70 cm), Bv and Mx showed a 38% higher root biomass compared to Lp, and there was a 43% increase under Bv LS defoliation frequency. Total root biomass (0 – 70 cm) was similar between species and defoliation frequencies, and the interaction was not statistically significant (Table 6.2).

Table 6.2. Accumulated herbage mass in the first and second year, and root biomass at the end of the second year for *B. valdivianus* (Bv) monoculture, *L. perenne* (Lp) monoculture and the mixture at Lp optimal leaf stage (LS) and Bv LS defoliation frequency.

	Accumulated herbage mass (kg/ha)		Root biomass (kg/m^3)			
	Year 1	Year 2	0–10 cm	11–30 cm	31–70 cm	0–70 cm
Pasture						
B. valdivianus	12184b	10929b	9.58	0.93 a	0.16 a	10.64
L. perenne	11614b	11938b	8.63	0.76 b	0.09 b	9.48
Mixture	14186a	13407a	9.92	0.74 b	0.13 ab	10.79
Significance	***	***	NS	*	*	NS
Defoliation frequency						
Lp LS	13084	12659	9.7	0.65 b	0.09 b	10.44
Bv LS	12239	13337	9.05	0.97 a	0.16 a	10.16
Significance	NS	NS	NS	***	**	NS
Pasture×Defoliation frequency						
B. valdivianus × Lp LS	11982	10437	10.79	0.62 b	0.09	11.51
B. valdivianus × Bv LS	12387	11221	8.37	1.24 a	0.23	9.76
L. perenne × Lp LS	12021	11659	7.98	0.67 b	0.08	8.72
L. perenne × Bv LS	11207	12217	9.27	0.86 b	0.11	10.24
Mixture × Lp LS	15250	13001	10.32	0.65 b	0.11	11.08
Mixture × Bv LS	13122	13813	9.51	0.83 b	0.15	10.49
Significance	NS	NS	NS	*	NS	NS

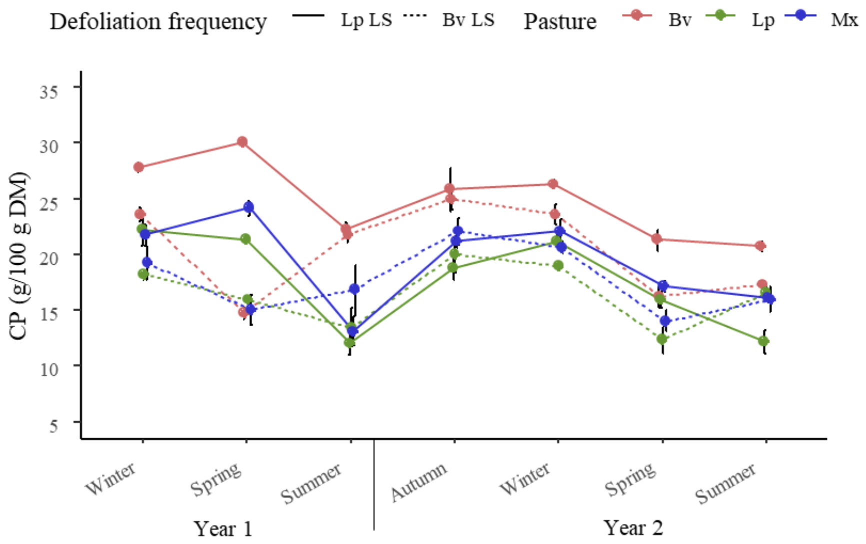
Letters that differ within columns indicate values that are significantly different at the following levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; NS, not significant ($p > 0.05$).

6.4.2 Herbage nutritive value

Overall, the content of CP, NDF, ADF, and OMD was mainly influenced by season within year ($p \leq 0.001$), followed by the pasture type ($p \leq 0.001$) and to a lesser extent (i.e., lower proportion of explained variation) by the defoliation frequency ($p \leq 0.001$). There was also a significant interaction between pasture type, defoliation frequency and season within year for the measured variables ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.01$, and $p \leq 0.001$, for CP, NDF, ADF and OMD, respectively) (Table 6.1).

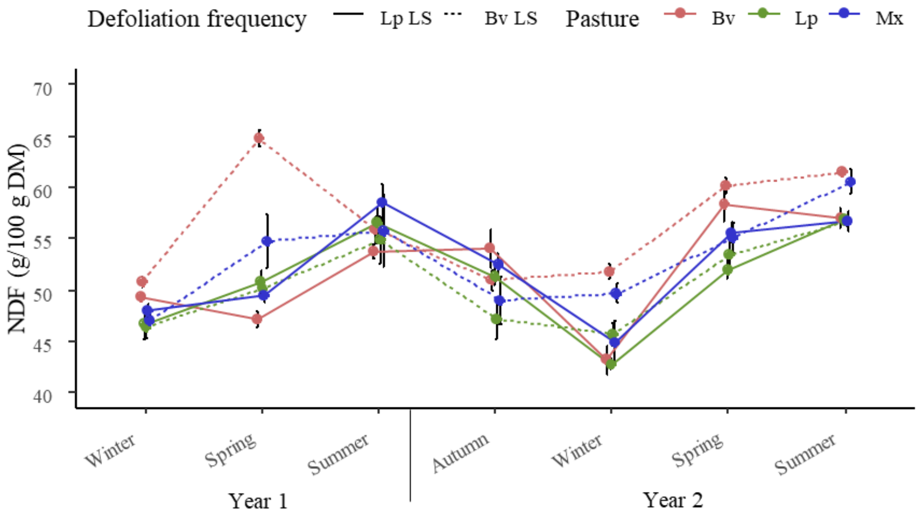
Except in summer of the first year and in autumn of the second year, when values were similar to Bv under Bv LS defoliation frequency, the highest CP values were in Bv under the high defoliation frequency (the highest level was ~ 30% DM in the first spring). In winter, the lowest CP value (~ 18% DM) was recorded in Lp and Mx (only in the second winter) under Bv LS defoliation frequency. Crude protein was consistently low under Bv LS defoliation frequency in all pasture types in the first spring, and in Lp and Mx in the second spring (reaching the lowest levels, ~ 12%). Whereas under Lp LS defoliation frequency there was a 32% increase for Lp and Mx. During summer, Mx and Lp presented similar values regardless of the defoliation frequency, except in the second summer where Lp showed the lowest CP content (~ 12% DM) under Lp LS defoliation frequency. Moreover, in autumn similar values were found as in the second winter, showing only a decrease in Bv under Bv LS defoliation frequency (Figure 6.4a).

a)



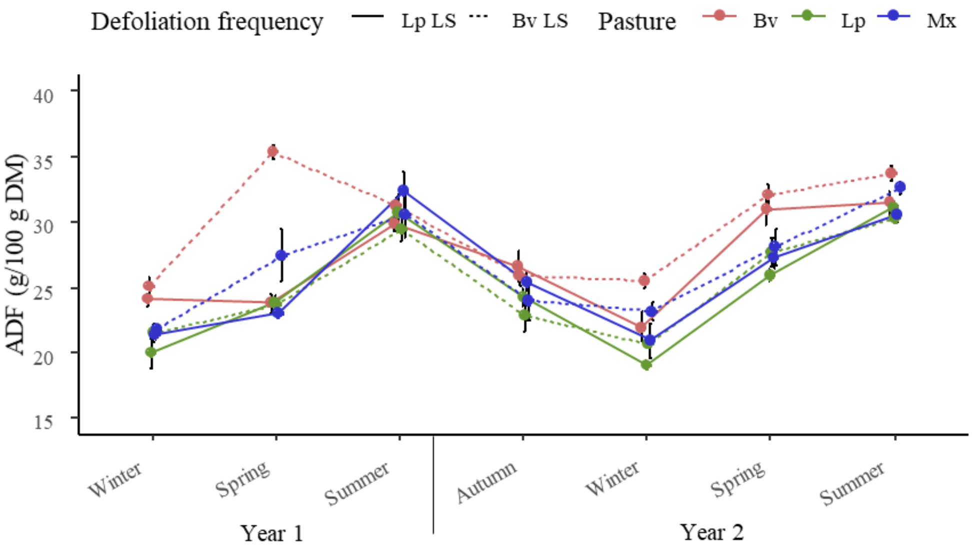
Defoliation frequency
Lp LS
Bv LS
Pasture
Bv
Lp
Mx
CP (g/100 g DM)
35
30
25
20
15
10
5
Winter
Spring
Summer
Autumn
Winter
Spring
Summer
Year 1
Year 2

b)



Defoliation frequency
Lp LS
Bv LS
Pasture
Bv
Lp
Mx
NDF (g/100 g DM)
70
65
60
55
50
45
40
Winter
Spring
Summer
Autumn
Winter
Spring
Summer
Year 1
Year 2

c)



Defoliation frequency
Lp LS
Bv LS
Pasture
Bv
Lp
Mx
ADF (g/100 g DM)
40
35
30
25
20
15
Winter
Spring
Summer
Autumn
Winter
Spring
Summer
Year 1
Year 2

d)

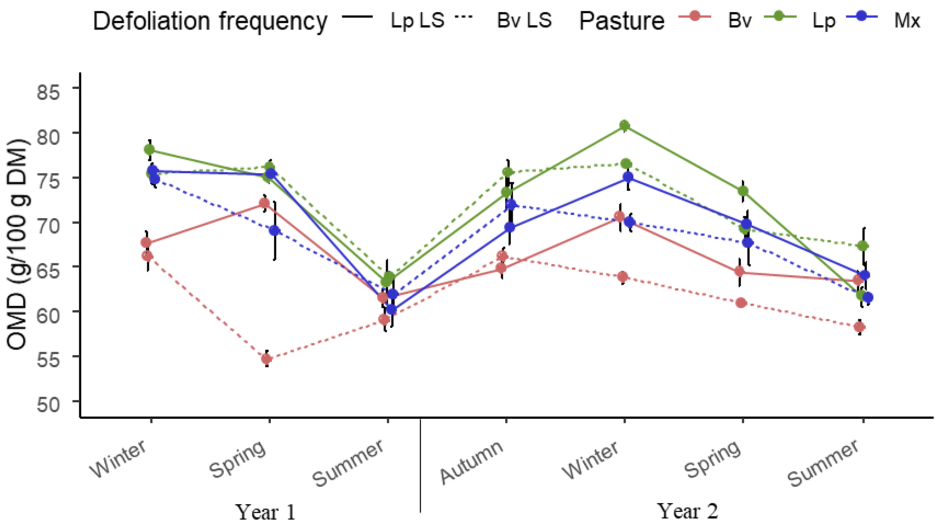


Figure 6.4. Interaction between Lp LS (solid line; based on *L. perenne* [Lp] optimal leaf regrowth stage [LS]) and Bv LS (dashed line; based on *B. valdivianus* [Bv] optimal leaf regrowth stage) defoliation frequency and pasture type (*Bromus valdivianus* monoculture [Bv], *Lolium perenne* [Lp], and mixture of both species [Mx]) across seasons for: a) crude protein (CP), b) neutral detergent fibre (NDF), c) acid detergent fibre (AFD), and c) organic matter digestibility (OMD). The standard errors of the mean are shown in vertical bars.

The NDF and ADF content followed similar seasonal trends. Overall, the pastures showed an increase in summer and spring, compared to winter and autumn. Under Bv LS defoliation frequency, Bv increased the amount of fiber in spring (showing the highest levels: 65% and 35% DM for NDF and ADF, respectively), winter and the second summer, reaching similar values to Bv under Lp LS defoliation frequency in the first winter and second spring and to Mx under Bv LS defoliation treatment during the second summer. On the other hand, NDF and ADF in Lp were similar under both defoliation treatments and were lower than Mx under Bv LS defoliation frequency in the spring of year one, the second winter, and the second summer (Figure 6.4b and c).

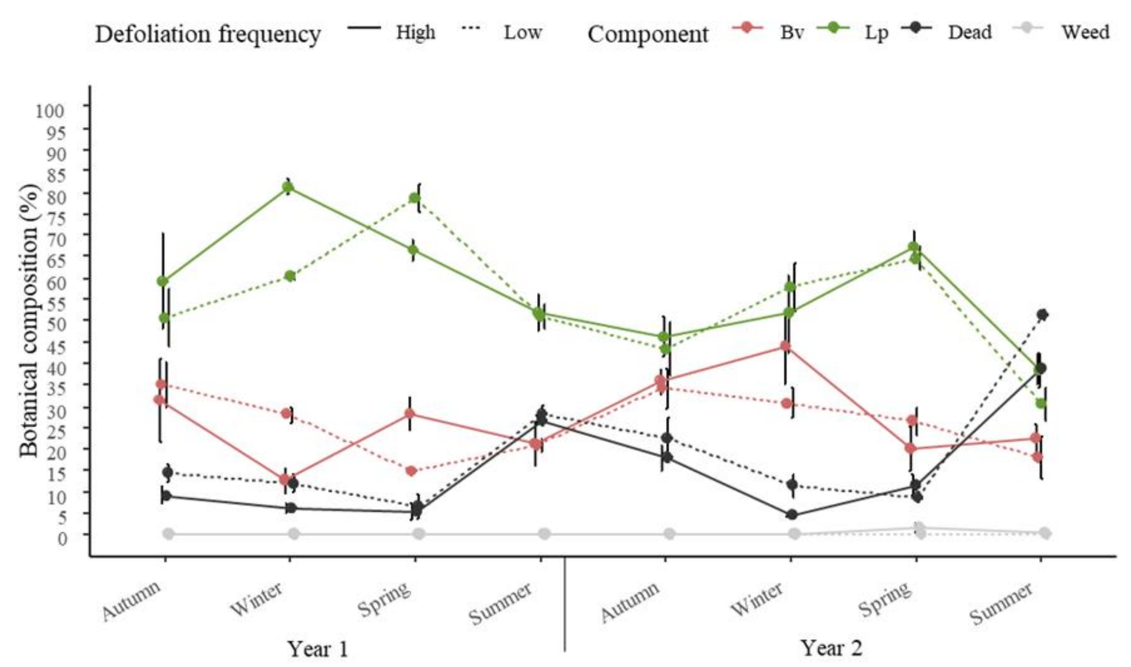
The OMD showed high fluctuations throughout the year, which were consistent when year 1 and year 2 were contrasted. In the summer period the OMD values diminished in all the pastures. *Bromus valdivianus* under the Bv LS defoliation frequency presented the lowest levels for OMD (reaching ~ 55% DM in first spring), except for the first winter and autumn when it was similar to Bv under Lp LS defoliation frequency, and the first summer when it was also similar to Mx.

The Lp under Lp LS defoliation frequency showed the highest OMD values in winter and spring of the second year (79% DM and 73% DM, respectively). However, it was similar with Mx under Lp LS and with Lp under Bv LS defoliation frequency in the rest of the seasons. The Mx under the Bv LS defoliation treatment in the first spring and second winter presented OMD values of ~ 70% DM that were similar to those of Bv at Lp LS defoliation frequency (Figure 6.4d).

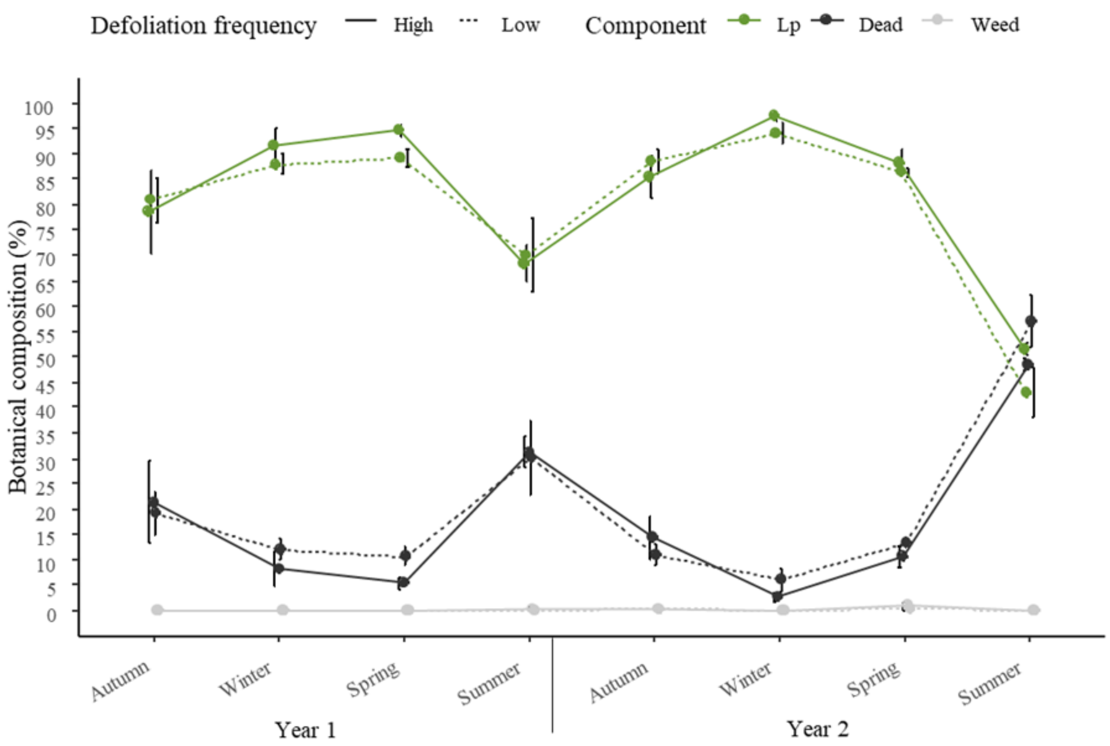
6.4.3 Seasonal change in botanical composition of pastures

Overall, the defoliation frequency treatments did not significantly change the botanical composition in the mixture and in the monocultures. In Mx, during the first year, Lp dominated the pasture, but it decreased by 28% in summer, while dead matter increased by 22% in summer and Bv decreased by 36% from autumn to summer. However, Bv proportion increased in the Mx from the first summer to the second autumn. The Bv in the Mx defoliated at Lp LS frequency obtained similar values to Lp in the winter of the second year. From the winter of the second year the pastures presented a similar trend to that of the first year, with an increase in Lp (dominating again the pastures) and a decrease in Bv in the spring; while during the summer the dead matter increased from 10% in spring to 51% (reaching the highest value) under Bv LS defoliation frequency. The Bv in the same summer maintained the same proportion as spring, but the Lp contribution decreased by 48% in the mixture (Figure 6.5a).

a)



b)



c)

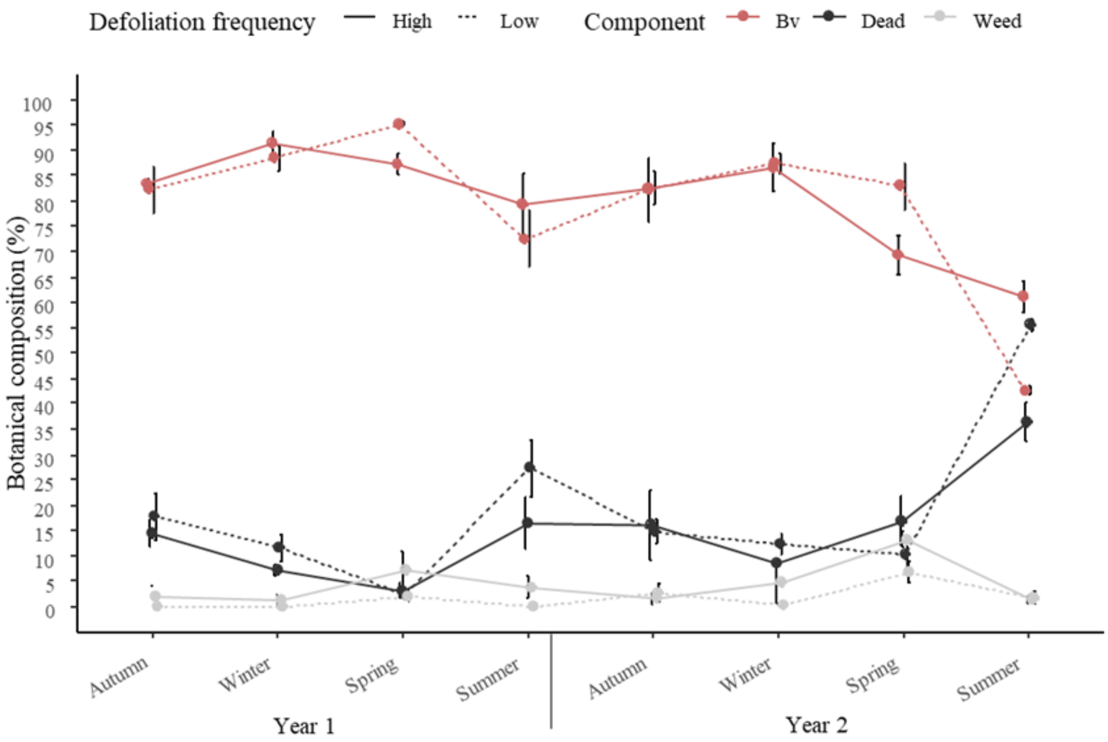


Figure 6.5. Botanical composition across the two years of evaluation for a) mixture of *L. perenne* (Lp) and *B. valdivianus* (Bv), b) *L. perenne* monoculture (Lp) and c) *B. valdivianus* monoculture (Bv). Pastures were defoliated at Lp LS defoliation (solid lines; based on *L. perenne* optimal leaf regrowth stage [LS]) and Bv LS defoliation frequencies (dashed line; based on *B. valdivianus* optimal leaf regrowth stage). Dead matter, dead; and species not sown, weed. The standard errors of the mean are shown in vertical bars.

The two monocultures also showed similar trends in the change of component proportions. Both species dominated the monocultures, only reaching a proportion below ~ 65% in the second summer of study. Summer conditions increased dead matter in the monocultures, with the highest value being reached in the second summer under the Bv LS defoliation frequency (~ 55%). In the first summer, Bv decreased by 17.5%, and Lp by 24.1%. During the second summer, Lp decreased by 51% and 42% for Bv LS and Lp

LS defoliation frequency; respectively. Whereas Bv decreased by 49% under Bv LS defoliation frequency and 12% at Lp LS defoliation frequency. It is important to note that the weed component was almost absent in Lp and Mx, but in Bv the proportion of weeds was not low, reaching values of 10% in the second spring (Figure 6.5b and c).

6.4.4 Tiller population dynamics

In the Mx pasture, the Bv tiller density was unaffected by defoliation frequency and was lower than Lp tiller density until March 2020, when tiller density of Lp defoliated at the Bv LS frequency was similar to Bv (Figure 6.6a). Tiller density was highest in Lp under Lp LS defoliation frequency, apart from short periods of time in summer, when it was similar to tiller density of Lp under Bv LS defoliation frequency. There was a steady decrease in tiller density in Lp over time, with a high decline during summer (62.7% and 31.7% decrease in tiller density, for Lp LS and Bv LS defoliation frequency, respectively) and a partial recovery afterwards (mainly under Lp LS defoliation frequency). Conversely, the tiller density of Bv, although lower, was more stable and showed a far smaller decline during late spring of both years, but the population recovered during summer to reach values similar to those in winter and early spring. Over the two-year study period, the average Lp tiller density in Mx pasture decreased from ~7300 tillers/m^2 to ~4400 tillers/m^2, whereas Bv increased from ~3000 tillers/m^2 to ~4000 tillers/m^2.

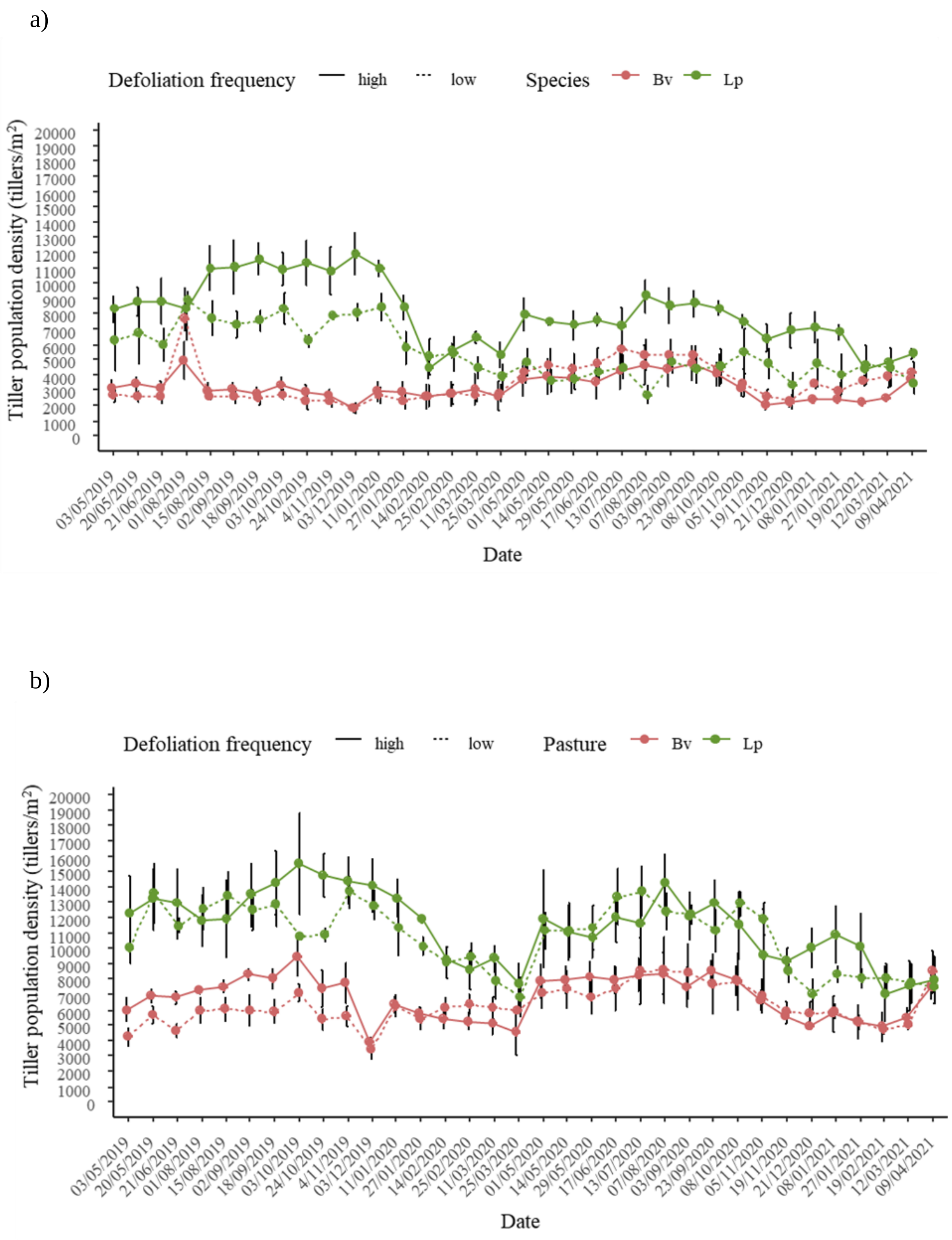


Figure 6.6. Tiller dynamics for: a) mixed pastures of *L. perenne* (Lp) and *B. valdivianus* (Bv), and b) monocultures of *L. perenne* (Lp) and *B. valdivianus* (Bv). The three pastures were defoliated to Lp optimal leaf stage [LS] (solid line) and Bv LS (dashed line) defoliation frequencies. The standard errors of the mean are shown in vertical bars.

Tiller densities in the monocultures (Figure 6.6b) exhibited similar overall trends to the Mx pasture, with no consistent effect of defoliation frequency, and higher tiller densities in Lp compared with Bv, again until March 2020 when Lp density declined to around the Bv tiller density values. From a peak in November 2019, there was a 50% decrease in Lp tiller density by the end of March 2020 (~ 14000 tillers/m^2 to ~ 7200 tillers/m^2, respectively). However, tiller density recovered during the following autumn and winter, before again steadily declining to the last summer (38% decrease in tiller density). Although Lp started with more than double the number of tillers than Bv at the beginning of the evaluation period, at the last evaluation date, both species had a similar number of tiller density (~ 7,800 tillers/m^2). From a peak in September 2019 around 8000 tillers/m^2, tiller density in Bv decreased by 56% in late spring (December 2019), followed by recovery firstly in January 2020 and then again in May 2020, to ~ 7600 tillers/m^2. Tiller density of Bv was then relatively stable until November 2020, when it began a gradual decline until December 2020, before again stabilising at around 5300 tillers/m^2 until a sharp increase in April 2021 to end the study with ~ 8000 tillers/m^2.

6.5 DISCUSSION

The current study highlighted the effect of mixing two perennial grass species (*B. valdivianus* and *L. perenne*) that differ in growth strategy. Primarily, it showed an enhancement in herbage mass production in the mixture compared to the monocultures. The superior yield of Mx could be mainly explained by the complementary growth patterns of Lp and Bv. During periods of high water availability in the soil (winter, and sometimes spring) Lp produced more herbage mass than Bv, while Bv produced higher herbage mass than Lp after the dry periods (i.e. autumn), which is associated with a drought-resistance strategy (Volaire, 2018). The defoliation frequency did not alter the growth of Mx in terms of production and persistence, as neither yield, botanical composition, nor number of tillers varied with defoliation frequency. This confirmed that the two species have an overlapping leaf stage ‘window’ that optimises production and persistence, making the decision on when to graze the mixture less complex.

6.5.1 Benefits of a mixture in term of accumulated herbage mass, seasonal herbage mass production and root biomass

Several studies have shown the higher biomass production of mixed pastures compared to monocultures, which was related to an increase in species diversity and contrasting functional traits in the mixed pastures (Finn et al., 2018; Suter et al., 2021; Tilman et al., 1997). The present study supported this mixture effect, but it also demonstrated that adding two perennial grasses that differ in their functional traits related to biomass allocation to root at depth can significantly improve herbage mass. This result contrasts with the findings of Suter et al. (2021) and Barkaoui et al. (2016), as they found no production improvement when mixing a shallow and deep-rooted species. In the case of the Suter et al. (2021) study, only when a legume was incorporated into the mixture of *L. perenne* and *D. glomerata* (chosen as the deeper root species) they found a greater stability (less production fluctuation across seasons) and a higher overall production. While Barkaoui et al. (2016), that found higher pasture resilience (assessed as the recovery degree following a drought) when mixing both type of species (*D. glomerata* and *Festuca arundinacea* Schreb were the deep-rooted species, and *Carex humilis* Leyss and *Festuca christianii-bernardii* Kerguélen the shallow-rooted species), they found no benefit of the mixed species on production. The difference between these studies and the present study could be attributed to either the use in the present study of Bv in the mixture, or to the implemented defoliation frequency strategy, or both. Fulkerson and Donaghy (2001) determined that a defoliation management based on leaf regrowth stage is a suitable approach to sustain high production without compromising pasture persistence, and harvesting the species when their energy reserves are replenished might allow them to express their functional traits, such as exploring soil at depth through root elongation (Donaghy et al., 2008; García-Favre et al., 2021).

In the present study it was shown that the mixture of two perennial grasses can improve the herbage mass through complementary growth over a period of two years, and even a synergist effect between the two species, as Mx out-produced Bv monoculture in the second summer (Hofer et al., 2016) (Figure 6.2). Although a two-fold production advantage was observed in a four species mixture (i.e. two grasses and two legumes) (Nyfeler et al., 2009), the increase of 15% in herbage mass production in the present study made it possible to assess the effect of mixing Lp and Bv under grazing and rainfed conditions. The species complementarity in spring allowed the mixture to produce more herbage mass than the monocultures due to different flowering times of the two species. Stem elongation marks the maximum growth rate in the growing season (Parsons, 1988), and cv. Trojan (classified as late heading time by Herridge et al. (2021)) produced a concentrated flowering in mid-December, whereas Bv started flowering in early November and continued up until early January (data not shown).

Pasture DM yield during the autumn season was low for all the pasture types, due to low rainfall and high evaporation during summer impacting on soil moisture levels. The summer season (December – February) started with a better soil water balance than the autumn season (March – May, Figure 6.1). Drought resistance/recovery of species can also be measured in their growth when soil water balance increases following a dry period (Finn et al., 2018). The greater yield of Bv monoculture compared with Lp monoculture during autumn could be attributed to a better response to rainfall, as plants may have increased WSC concentration in the stubble during the dry period (García-Favre et al., 2021), facilitating a faster growth following the dry period (Volaire et al., 1998). In addition, Bv probably harvested more water from deep soil layers (Ordóñez et al., 2018), while water was scarce in top layers, due to higher root biomass at depth, thus maintaining the plants in a more active growth state during the period of water shortage and as part of a dehydration avoidance strategy (Zwicke et al., 2015). Barkaoui et al. (2016) stated that greater water uptake from deep soil layers increased pasture resilience but did not lead to an increase in herbage production during the period of water scarcity. This improved pasture resilience in Mediterranean pastures was related to greater herbage yield the following spring, while in the present study, due to different climatic conditions, the effect on pasture production was observed in the autumn. The same logic can be used to explain the higher yield in autumn of Mx compared to Lp, however, and interestingly, the production of Mx in this period was similar to that of Bv. This indicates that it was the presence of Bv in the Mx pasture, with proportion values increasing in autumn up to 40% of the total botanical composition, that drove the higher herbage yield.

During winter when the temperature was lower and the soil moisture levels were higher (Figure 6.1), Lp out-yielded Bv, and this could be mainly explained by the limited growth of Bromus genus when the soil contains lower levels of oxygen (Stewart, 1996). The better response of Lp to higher soil moisture conditions was probably a result of the presence of cultivar-specific mechanisms (McFarlane et al., 2003), such as formation of aerenchymas in the root cortex and/or adventitious root development (Ashraf, 2012).

Root biomass accumulation was evaluated at the end of the study (i.e., March 2021) and in contrast to the increased herbage mass production, the Mx root system was not significantly larger than either Bv or Lp root systems. The root development seemed to be a species-specific response, as there was no additional root accumulation in Mx due to a lack of niche complementarity between species (Mommer et al., 2010). Although the present results were consistent with what other studies had estimated for the greater water uptake by Bv root systems (Ordóñez et al., 2018) from deep soil layers, it would have been preferable to study the root growth dynamics throughout the year. Such a future study would assess the periods of the year where a more infrequent grazing may result in a deeper soil exploration and greater water uptake from deep soil layers under drought conditions.

6.5.2 Variation in botanical composition and tiller dynamics

Grazing increases species richness by reducing competition among species, but only when grazers select the most competitively dominant species in the community (Michaels et al., 2021); however, grazing frequency might benefit one species at the expense of another. In the present study, no change in the botanical composition was observed, and this was expected, as within both defoliation frequency treatments, both Lp and Bv were within their optimal LS. Therefore, competition between species was not modified by defoliation management, and the overall botanical composition was the result of the greater competitive ability of Lp (Teughels et al., 1995) and the interaction between genotype (species) and environment. The only season when both species had similar proportions in the Mx (there was minimal variations between defoliation treatments) was during the second autumn, reflecting the better recovery and competition for resources of Bv from a dry summer/early autumn (Figure 6.5a). However, this botanical composition was reversed in the following seasons, as Lp exhibited a better fitness under well-watered conditions (López et al., 2013; Zhao et al., 2017).

The number of tillers per area is one pasture component that contributes to overall yield (Chapman and Lemaire 1993), and the steady replacement of live tillers ensures pasture persistence (Briske & Noy-Meir, 1998). In the present study, regardless of pasture type and defoliation frequency treatments, contrasting differences were found between the species. Although Bv showed a similar seasonal pattern of change in tiller density to Lp, in both monoculture and Mx, the final tiller density of Lp was lower than at the start of the study, whereas the final tiller density of Bv increased in the monoculture and in Mx, compared with the start of the study. Similar results were found by Woodward et al. (2020), who reported a decrease in Lp persistence due to death of tillers over the summer, which severely compromised the longevity of the pasture. In the present study, there was a sharp decrease in the Bv tiller density in late spring, as a result of simultaneous processes occurring within the canopy: shading (due to stem elongation) and a low red/far red ratio reducing tillering (Gastal & Lemaire, 2015), and the reduction or cessation of the growth of secondary tillers due to the additional demand for resources created by inflorescence development in the parent tillers (Briske & Noy-Meir, 1998). However, the Bv tiller density recovered during summer/early autumn under an environment of low soil water content, which indicated the better fitness of Bv to survive summer drought conditions. In addition, Volaire et al. (1998) found a high correlation between tiller survival during summer and pasture growth recovery after it, which is consistent in the present study with the higher herbage production of Bv and Mx than Lp during autumn. This finding aligns with the results of Hoekstra et al. (2014) that deep-rooted species were more drought resistant. The present study showed that based on the maintenance of tiller population over the study period, Bv was a more drought-resistant species than Lp.

6.5.3 The nutritive value of pastures

The determination of herbage nutritive value is crucial to determine feeding value for grazing stock. *Lolium perenne* is frequently used as a research standard to compare with other species (Bruinenberg et al., 2002), as its production and herbage quality have been assessed in a large range of environments (Fulkerson et al., 2007). Under dryland conditions in Australia, the NDF and CP levels were lower, and ME higher, than *Bromus willdenowii* Kunth. and *D. glomerata* (Turner et al., 2006). In the present study, seasonal variation in the nutritive value of the pastures explained more of the variation than pasture type and defoliation frequency treatments (Table 6.1). The differences in nutritive value between species were close to those reported by Calvache et al. (2020) in the south of Chile, with the herbage quality of Mx either similar to Lp, or in between Lp and Bv pastures. Throughout the study, Lp had higher OMD values (i.e., digestibility and therefore energy density of the forage) while Bv (mainly under Lp LS defoliation frequency) had the highest CP concentrations (Figure 6.4), however, these values varied across seasons. During the first summer, all the pastures presented similar values for OMD (which was low), ADF and NDF (which were both high). This pattern over summer, and the inverse relationship between fibre (ADF, NDF) and digestibility (OMD) was expected, as warmer temperatures result in more structural fibrous material, and these higher fibre levels in herbage reduce its digestibility (Roche et al., 2009). The generally higher fibre content in Bv pastures throughout the present study may be related to the greater tiller size of Bv compared to Lp (López et al., 2013), which could require more fibre to sustain this larger size, however, this assertion needs to be tested.

The higher fibre content of Bv in spring was related to more extensive stem elongation than Lp, with a greater number of Bv tillers becoming reproductive over a longer period (data not shown), and this could constrain animal production. However, a small increase in defoliation frequency (by only ½ a LS) during late spring prevented a sharp decline in herbage nutritive value. This broadly agrees with results obtained by Turner et al. (2006) for *B. willdenowii* in a drier environment under cutting, although their increase in herbage quality was far smaller (only 0.2 MJ higher metabolisable energy), and their increase in defoliation frequency larger (from 3 or 4 leaves/tiller, to 2 leaves/tiller), than in the present study. Turner et al. (2006) postulated that the more frequent defoliation required to maintain higher quality herbage in *Bromus* species might impact negatively on plant persistence due to exhaustion of WSC reserves, however in the current study, the small increase in defoliation frequency implemented, within the range previously defined as optimal for growth and persistence (García-Favre et al., 2021), appeared to have no negative implications. A slightly faster defoliation interval, especially in the spring when growth is fast and tillers are reproductive, might have the added benefit of disrupting reproductive development (thereby preventing apical dominance) and increasing light into the base of the pasture (thereby stimulating tillering), without a significant decline in WSC, however this will need to be tested more thoroughly.

6.6 CONCLUSIONS

The results of the present study demonstrated that the design of intensively managed pasture compositions under rainfed conditions should prioritise the asynchrony of species growth and the maximisation of deep root biomass. Therefore, in the mixture Lp grew better during periods with low temperatures and high soil moisture levels (e.g., winter), whereas Bv displayed greater growth during the recovery period after a drought. In spring, there was complementary growth between species.

Defoliation frequency treatments did not modify accumulated herbage mass production in the pastures, showing the overlapping of the species optimal LS (both species where within their optimal LS range), with ½ a LS different from the two species. However, Lp LS defoliation frequency decreased root biomass accumulation at deep soil layers, which could threaten Bv persistence in the following years and thus impair the functionality of the species. The caveat here is that the use of Bv LS defoliation frequency showed low nutritive value in Bv during spring, which could limit animal performance. In Mx the nutritive value was higher and closer to Lp monoculture. Although, to ensure a high nutritive value throughout the year and at the same time maintain a large root biomass at depth in Mx, a flexible grazing management (i.e., changing defoliation frequency based on LS of the species) should be used. That is, during winter and early/mid spring the defoliation should be based on the optimal LS of Lp, while in late spring, summer, and autumn (when soil water is scarce) it should be based on the optimal LS of Bv. This assertion requires investigation as the root growth pattern across seasons for Bv is unknown.

6.7 REFERENCES

Ashraf, M. A. (2012). Waterlogging stress in plants: A review. *African Journal of Agricultural Research*, *7*(13), 1976-1981.

Barkaoui, K., Roumet, C., & Volaire, F. (2016). Mean root trait more than root trait diversity determines drought resilience in native and cultivated Mediterranean grass mixtures. *Agriculture, Ecosystems & Environment*, *231*, 122-132.

Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., . . . Yang, L. H. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, *508*(7497), 517-520.

Briske, D., & Noy-Meir, I. (1998). Plant responses to grazing: a comparative evaluation of annual and perennial grasses. *Ecological basis of livestock grazing in Mediterranean ecosystems*, *13*, 26.

Bruinenberg, M., Valk, H., Korevaar, H., & Struik, P. (2002). Factors affecting digestibility of temperate forages from seminatural grasslands: a review. *Grass and Forage Science*, *57*(3), 292-301.

Cai, J., Liu, Y., Lei, T., & Pereira, L. S. (2007). Estimating reference evapotranspiration with the FAO Penman–Monteith equation using daily weather forecast messages. *Agricultural and forest meteorology*, *145*(1-2), 22-35.

Calvache, I., Balocchi, O., Alonso, M., Keim, J. P., & F. López, I. (2020). Thermal Time as a Parameter to Determine Optimal Defoliation Frequency of Perennial Ryegrass (*Lolium perenne* L.) and Pasture Brome (*Bromus valdivianus* Phil.). *Agronomy*, *10*(5).

Campbell, A. (1966). Grazed pasture parameters. I. Pasture dry-matter production and availability in a stocking rate and grazing management experiment with dairy cows. *The Journal of Agricultural Science*, *67*(2), 199-210.

Corson, D., Waghorn, G., Ulyatt, M., & Lee, J. (1999). NIRS: Forage analysis and livestock feeding. Proceedings of the New Zealand Grassland Association,

Deru, J., Schilder, H., van der Schoot, J. R., & van Eekeren, N. (2014). Genetic differences in root mass of Lolium perenne varieties under field conditions. *Euphytica*, *199*(1), 223-232.

Descalzi, C., Balocchi, O., López, I., Kemp, P., & Dörner, J. (2018). Different soil structure and water conditions affect the growing response of *Lolium perenne* L. and *Bromus valdivianus* Phil. growing alone or in mixture. *Journal of soil science and plant nutrition*, *18*(3), 617-635.

Diaz, S., Lavorel, S., McIntyre, S., Falczuk, V., Casanoves, F., Milchunas, D. G., . . . Noy-Meir, I. (2007). Plant trait responses to grazing–a global synthesis. *Global Change Biology*, *13*(2), 313-341.

Donaghy, D. J., & Fulkerson, W. J. (1998). Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and Forage Science*, *53*, 211-218.

Donaghy, D. J., Turner, L. R., & Adamczewski, K. A. (2008). Effect of defoliation management on water-soluble carbohydrate energy reserves, dry matter yields, and herbage quality of tall fescue. *Agronomy Journal*, *100*(1).

Duru, M., Cruz, P., Ansquer, P., & Navas, M. L. (2014). Standing herbage mass: An integrated indicator of management practices for examining how fertility and defoliation regime shape the functional structure of species-rich grasslands. *Ecological Indicators*, *36*, 152-159.

Finn, J. A., Suter, M., Haughey, E., Hofer, D., & Lüscher, A. (2018). Greater gains in annual yields from increased plant diversity than losses from experimental drought in two temperate grasslands. *Agriculture, Ecosystems & Environment*, *258*, 149-153.

Fulkerson, W., Slack, K., & Lowe, K. (1994). Variation in the response of Lolium genotypes to defoliation. *Australian Journal of Agricultural Research*, *45*(6), 1309-1317.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture*, *41*, 261-275.

Fulkerson, W. J., Neal, J. S., Clark, C. F., Horadagoda, A., Nandra, K. S., & Barchia, I. (2007). Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. *Livestock Science*, *107*(2-3), 253-264.

García-Favre, J., López, I. F., Cranston, L. M., Donaghy, D. J., & Kemp, P. D. (2021). The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels. *Agronomy, 11*(2).

Gastal, F., & Lemaire, G. (2015). Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: review of the underlying ecophysiological processes. *Agriculture*, *5*(4), 1146-1171.

Gatti, M. L., Ayala Torales, A. T., Cipriotti, P. A., & Golluscio, R. A. (2017). Effects of defoliation frequency and nitrogen fertilization on the production and potential for persistence of Dactylis glomerata sown in multispecies swards. *Grass and Forage Science*, *72*(3), 489-501.

Grace, C., Boland, T. M., Sheridan, H., Brennan, E., Fritch, R., & Lynch, M. B. (2019). The effect of grazing versus cutting on dry matter production of multispecies and perennial ryegrass-only swards. *Grass and Forage Science*, *74*(3), 437-449.

Grime, J. P., Hodgson, J. G., & Hunt, R. (2014). *Comparative plant ecology: a functional approach to common British species*. Springer.

Henessy, K., Fitzharris, B., Bates, N., Harvey, S., Howden, M., Hughes, L., Salinger J., & Warrick, R. (2007). Australia and New Zealand, climate change 2007: Impacts, adaptation and vulnerability. In M. Parry, O. Canzani, J. Palutikof, P. van der Linden, & C. Hanson (Eds.), *Contributions of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Herridge, R., Brownfield, L., & Macknight, R. (2021). Identification and Characterization of Perennial Ryegrass (*Lolium perenne*) vernalization genes. *Frontiers in plant science*, *12*, 351.

Hewitt, A. E. (2010). New Zealand soil classification. *Landcare research science series*(1).

Hoekstra, N. J., Suter, M., Finn, J. A., Husse, S., & Lüscher, A. (2014). Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? *Plant and Soil, 394*(1-2), 21-34.

Hofer, D., Suter, M., Haughey, E., Finn, J. A., Hoekstra, N. J., Buchmann, N., . . . Bennett, J. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology*, *53*(4), 1023-1034.

Husse, S., Huguenin-Elie, O., Buchmann, N., & Lüscher, A. (2016). Larger yields of mixtures than monocultures of cultivated grassland species match with asynchrony in shoot growth among species but not with increased light interception. *Field Crops Research*, *194*, 1-11.

Keim, J. P., López, I. F., & Balocchi, O. A. (2015). Sward herbage accumulation and nutritive value as affected by pasture renovation strategy. *Grass and Forage Science*, *70*(2), 283-295.

Kerr, G. A., Chapman, D. F., Thom, E. R., Matthew, C., Van Der Linden, A., Baird, D. B., ... & Corkran, J. R. (2012, January). Evaluating perennial ryegrass cultivars: improving testing. In *Proceedings of the New Zealand Grassland Association* (pp. 127-135).

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software, 82*(13), 1-26.

Lee, J., Thom, E., Waugh, C., Bell, N., McNeill, M., Wilson, D., & Chapman, D. (2017). Trajectory and causes of decline in the botanical composition of dairy-grazed pasture in the Waikato. *Journal of New Zealand Grasslands*, 89-96.

Lee, J. M., Matthew, C., Thom, E. R., & Chapman, D. F. (2012). Perennial ryegrass breeding in New Zealand: A dairy industry perspective. *Crop and Pasture Science*, *63*(2), 107-127.

Lenth, R. V. (2017). Using lsmeans. *Journal of Statistical Software 69*, 1-33.

López, I., Balocchi, O., Lailhacar, P., & Oyarzun, C. (1997). Characterization of the growing sites of six native and naturalized pasture species in the Dominio Humedo of Chile. *Agro Sur 25*, 62-80.

López, I. F., Kemp, P. D., Dörner, J., Descalzi, C. A., Balocchi, O. A., & García, S. (2013). Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. and *Lolium perenne* L. plants under water restriction. *Journal of Agronomy and Crop Science*, *199*(6), 449-459.

McFarlane, N., Ciavarella, T., & Smith, K. F. (2003). The effects of waterlogging on growth, photosynthesis and biomass allocation in perennial ryegrass (*Lolium perenne* L.) genotypes with contrasting root development. *The Journal of Agricultural Science*, *141*(2), 241-248.

Michaels, J., Batzer, E., Harrison, S., & Eviner, V. T. (2021). Grazing affects vegetation diversity and heterogeneity in California vernal pools. *Ecology*, *102*(4), e03295.

Mommer, L., Van Ruijven, J., De Caluwe, H., Smit-Tiekstra, A. E., Wagemaker, C. A., Joop Ouborg, N., . . . De Kroon, H. (2010). Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. *Journal of Ecology*, *98*(5), 1117-1127.

Narasimhan, B., & Srinivasan, R. (2005). Development and evaluation of soil moisture deficit index (SMDI) and evapotranspiration deficit index (ETDI) for agricultural drought monitoring. *Agricultural and forest meteorology*, *133*(1-4), 69-88.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology, 46*(3), 683-691.

O'Mara, F. P. (2012). The role of grasslands in food security and climate change. *Annals of Botany*, *110*(6), 1263-1270.

Ordóñez, I., López, I. F., Kemp, P. D., Descalzi, C. A., Horn, R., Zúñiga, F., . . . Dörner, J. (2018). Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. *Soil and Tillage Research*, *178,* 55-64.

Ordóñez Vásquez, I. P. (2020). *Pursuing pasture tolerance and resilience through species with different functional traits and soil-plant-water interactions: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science at Massey University, Manawatu, New Zealand* (Doctoral dissertation, Massey University).

Parsons, A. (1988). The effects of season and management on the growth of grass swards. In *The grass crop* (pp. 129-177). Springer.

Pembleton, K. G., Tozer, K. N., Edwards, G. R., Jacobs, J. L., & Turner, L. R. (2015). Simple versus diverse pastures: opportunities and challenges in dairy systems. *Animal Production Science, 55*(7), 893-901.

Pimm, S. L. (1984). The complexity and stability of ecosystems. *Nature, 307*(5949), 321-326.

Pontes, L. d. S., Carrère, P., Andueza, D., Louault, F., & Soussana, J.-F. (2007). Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. *Grass and Forage Science, 62*(4), 485-496.

Roche, J., Turner, L., Lee, J., Edmeades, D., Donaghy, D., Macdonald, K., . . . Berry, D. (2009). Weather, herbage quality and milk production in pastoral systems. 2. Temporal patterns and intra-relationships in herbage quality and mineral concentration parameters. *Animal Production Science, 49*(3), 200-210.

Roscher, C., Kutsch, W., & Schulze, E. D. (2011). Light and nitrogen competition limit *Lolium perenne* in experimental grasslands of increasing plant diversity. *Plant Biology, 13*(1), 134-144.

Sanderson, M., Goslee, S., Soder, K., Skinner, R., Tracy, B., & Deak, A. (2007). Plant species diversity, ecosystem function, and pasture management—a perspective. *Canadian Journal of Plant Science, 87*(3), 479-487.

Senarath, A. (2003). *Soil spatial variability in northern Manawatu, New Zealand: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Soil Science at Massey University, Palmerston North, New Zealand* (Doctoral dissertation, Massey University).

Stewart, A. (1996). Potential value of some Bromus species of the section Ceratochloa. *New Zealand Journal of Agricultural Research, 39*(4), 611-618.

Suter, M., Huguenin-Elie, O., & Luscher, A. (2021). Multispecies for multifunctions: combining four complementary species enhances multifunctionality of sown grassland. *Sci Rep, 11*(1), 3835.

Team, R. C. (2013). R: A language and environment for statistical computing.

Teughels, H., Nijs, I., Van Hecke, P., & Impens, I. (1995). Competition in a global change environment: the importance of different plant traits for competitive success. *Journal of Biogeography*(22), 297-305.

Tilman, D. (2001). Functional diversity. *Encyclopedia of biodiversity, 3*(1), 109-120.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science*, *277*(5330), 1300-1302.

Turner, L., Donaghy, D., Lane, P., & Rawnsley, R. (2006). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 2. Nutritive value. *Grass and Forage Science*, *61*(2), 175-181.

Vogel, A., Scherer-Lorenzen, M., & Weigelt, A. (2012). Grassland resistance and resilience after drought depends on management intensity and species richness. *PLoS One, 7*(5), e36992.

Volaire, F. (2018). A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Glob Chang Biol, 24*(7), 2929-2938.

Volaire, F., Thomas, H., & Lelièvre, F. (1998). Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: I. Growth, death, water relations and solute content in herbage and stubble. *New Phytologist, 140*(3), 439-449.

Woodward, S. J. R., Van Oijen, M., Griffiths, W. M., Beukes, P. C., & Chapman, D. F. (2020). Identifying causes of low persistence of perennial ryegrass (*Lolium perenne*) dairy pasture using the Basic Grassland model (BASGRA). *Grass and Forage Science*, *75*(1), 45-63.

Zhao, Y., Li, Z., Zhang, J., Song, H., Liang, Q., Tao, J., . . . Liu, J. (2017). Do shallow soil, low water availability, or their combination increase the competition between grasses with different root systems in karst soil? *Environmental Science and Pollution Research, 24*(11), 10640-10651.

Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud'homme, M. P., & Volaire, F. (2015). What functional strategies drive drought survival and recovery of perennial species from upland grassland? *Ann Bot*, *116*(6), 1001-1015.

CHAPTER 7

General discussion

7.1 GRAZING OPPORTUNITY AND THE IMPORTANCE FOR FUNCTIONAL TRAITS IN PASTURES

Grazing management, defined by Allen et al. (2011) as “the manipulation of grazing in pursuit of a specific objective or set of objectives”, has been shown as a tool to preserve species persistence, pasture production and, thus, animal production (Clark, 2011; Jaurena et al., 2021). Different grazing strategies have been discussed in the literature, such as continuous (adjusting stocking rate according to pasture growth rate and pasture allowance) or rotational stocking, and it was proved that both strategies are feasible options in pastoral systems (Parsons et al., 1988), but with greater efficiency in herbage use in rotational stocking under conditions of low herbage growth (i.e., higher efficiency of harvested herbage) (Lemaire et al., 2009). The choice of strategy utilised depends mainly on the management that suits the logistics and infrastructure on a given farm. However, there are approaches that establish the grazing opportunity according to the objective pursued (i.e., optimise animal intake or pasture persistence) and the morphophysiological knowledge (i.e., leaf lifespan, pattern of energy reserve replenishment after defoliation) of the species composing the pastures. That is, pasture defoliation can be performed based on pasture height, pre-grazing herbage mass target, and leaf regrowth stage. The latter approach can only be performed if considerable studies on the accumulation of water-soluble carbohydrates at the tiller base (first 4–5 cm of the tiller base for temperate grass species), according with number of fully developed leaves, have been carried out (Donaghy et al., 2021). Fulkerson and Donaghy (2001) asserted that for pastures composed mainly of one grass species (*L. perenne* monoculture or 70:30% pasture of *L. perenne* and *T. repens*, respectively) leaf regrowth stage is the most appropriate defoliation criteria to enhance pasture growth and persistence. Therefore, it is necessary to assess the growth response of the species exposed to different defoliation frequencies based on the leaf regrowth stage. To date this has been performed in widely used species, such as *L. perenne* (Donaghy & Fulkerson, 1998), *Festuca arundinacea* Shrub. (Donaghy et al., 2008), and *Bromus willdenowii* Kunth. (Turner et al., 2006), under controlled conditions (glasshouse) and monocultures pastures. In Chapter 3, the defoliation for *B. valdivianus* (García-Favre et al., 2021) was set according to its leaf regrowth stage, which enhanced its herbage and root growth. In addition, in the present thesis, its growth throughout the year, herbage nutritive value and competition with *L. perenne* were assessed.

Although the aforementioned studies were conducted on pastures composed of a single species, the effect of species diversity on pasture production and persistence has recently been extensively investigated. Studying defoliation frequency effect (based on leaf regrowth stage) on species growth is an important first step but should be complemented by the study of the effect of neighbour competition on growth and the display of functional traits of the species. The influence of functional diversity in the ecosystem and how plant functional groups positively affect production and persistence of a pasture have been studied (Nyfeler et al., 2009; Tilman et al., 1997). However, the likelihood of functional plant traits development, which are the main driver of species performance during stressful conditions (e.g., low soil water level), could be limited by neighbour competition and the species management (i.e., defoliation frequency and intensity). Harvesting of plant organs by animals is a disturbance that produces a remobilisation of energy reserve (water-soluble carbohydrates [WSC]) from the tiller base (or stubble) to restore the photosynthetic apparatus (Fulkerson & Donaghy, 2001); but this disturbance also has implications on the competition for resources and species succession in diverse pasture. The effect of defoliation frequency, based on leaf regrowth stage, on the display of species functional traits and thus on competition for resource acquisition with neighbours has been little addressed in the literature. Therefore, the objective of Chapter 4 (García-Favre et al., 2021) was to assess how functional traits of *B. valdivianus* were modified by the defoliation frequency and neighbour presence (*L. perenne* in this case) under low and high soil water availabilities.

In the Grime model (CSR-model) framework, species are classified as ruderals, competitors, and stress tolerant (Grime et al., 2014). Although perennial grasses widely used in temperate regions for feeding ruminants share the three strategies of the CSR-model, there is one strategy that prevails over the other two depending on the environment (i.e., intensities of stress and disturbance) and how competitive the species is (in terms of resource acquisition). *Lolium perenne* has fast growing traits (i.e., fast above and below growth rates) which enhances resource acquisitions and strong competition with neighbours (Reich & Cornelissen, 2014). Whereas *B. valdivianus* has shown the ability to produce a great amount of herbage mass while displaying traits associated with species in the spectrum of slow traits, such as root biomass accumulation at depth and low tiller disappearance (high stability) throughout the year (Chapter 6). Therefore, mixing both species is an opportunity to increase resource utilisation through differentiation in niche exploration between the species, although, the dependence of this process on defoliation management is not fully understood in the literature. In Chapter 6, it was shown that *L. perenne* and *B. valdivianus* were able to explore different niches and thus complement each other throughout the year. However, the defoliation frequency based on optimal leaf regrowth stage (grazing opportunity), was a crucial tool to enhance herbage production, root growth and tiller survival of the pasture as it allowed the functional trait of species to develop (Chapter 3, 4, and 6). In addition, defoliation based on optimal leaf regrowth stage probably maintained low competition for resources between the species (Chapter 6). The mixture of *L. perenne* and *B. valdivianus* benefited from the intrinsic characteristics of both species and, thus, from the growth asynchrony across seasons (discussed in Chapter 6), which was also reported by Husse et al. (2016).

In the case of the *B. valdivianus* and *L. perenne* mixture, defoliation frequency based on the optimal leaf regrowth stage for either species did not modify overall herbage mass production, probably due to close window opportunity between both defoliation frequencies and/or a “homeostatic” mechanism in which change in tiller population and tiller size could act as a compensatory mechanism (Gastal & Lemaire, 2015). In Chapter 6, the environment and the intrinsic ability of species to compete had a higher influence on the botanical composition within the pastures, closely related to where each species is located within CSR-model. *Lolium perenne* had a higher presence in the pastures, as herbage mass component and as number of tillers, which indicated its high competitor strategy; it is an exploitative species following the definition by Cruz (2002). Whereas *B. valdivianus* showed a greater stress-tolerant strategy, due to maintenance of tiller population throughout the year, even during conditions of low water availability (Chapter 6). Increasing and diminishing their population (more pronounced in *L. perenne*) in the pasture showed that throughout the year different levels of mechanisms and/or different mechanisms of competition and stress tolerance are carried out. It is probably that different species present different mechanism through morpho-physiological responses to neighbours and the environment. Therefore, each mechanism/species could play a key role in the production and persistence of diverse pastures.

Functional groups diversity (i.e., the nature of the species, see redundancy hypothesis explained by Johnson et al. (1996)) increases production, resilience, and persistence of mixed pastures. Niche exploitation and, thus, resource use by the mixture of grass, legumes and forbs are well documented (e.g., Nyfeler et al. (2009), Hofer et al. (2016)). The enhancement of ecosystem performance is due to the functional roles of species in the community (Tilman et al., 1997), and interspecific competition for resources can impair the ecosystem benefits from species diversity (Johnson et al., 1996). The seasonality of resource (e.g., nitrogen, water, light) availability occurring in most of the farming systems around the world causes an alternation in the dominance of one species over another in mixed pastures (McNaughton, 1977). This could be modified by a disturbance, such as a defoliation event, altering the species relationship and thus resetting the competitive dominance of one species relative to another (Grime et al., 2014). In this way, there is continual feedback between environment, species and disturbances that drives ecosystems.

To alter species relationships (e.g., competition) and their growth dynamics in the community, the species used in the community (mainly in artificial pastures) and type and level of disturbance applied (disturbances related to anthropogenetic actions) can be modified. Therefore, different species and different disturbances (e.g., defoliation frequency) will have dissimilar outcomes in terms of ecosystem stability, resistance, and resilience in a particular system. In Chapter 6, the growth dynamic of two perennial grass species was addressed, one with a more exploitative strategy (*L. perenne*) and other with a more conservative strategy (*B. valdivianus*), competing for resources and under abiotic (drought) and biotic (defoliation) disturbances. As a result, in the mixed pasture, functional complementarity was enhanced by growth asynchrony throughout the year and different functional traits related to plant morphology (mainly root biomass at deep layers) between the species, which resulted in greater herbage production and could enhance pasture resistance and resilience (Volaire et al., 2014).

7.2 SEASONAL ROOT GROWTH, WHAT IS KNOWN ABOUT CARBON SUPPLY TO ROOTS TO PROMOTE SOIL EXPLORATION; CAN DEFOLIATION FREQUENCY ENHANCE ROOT PRODUCTION?

Climate change is challenging New Zealand farming systems, with a marked decrease in summer production (in some regions) and pasture persistence due to *L. perenne* tiller deaths (Woodward et al., 2020). Review articles have discussed how to maximise herbage mass growth rate and pasture utilisation (mainly in *L. perenne* pastures) (Chapman, 2016; Gastal & Lemaire, 2015), and increase summer herbage production with more summer-active species (although, with little production during cold months), such as, *Plantago lanceolata* L., *Cichorium intybus* L. (Pembleton et al., 2015). However, in New Zealand, a different approach can be undertaken to enhance pasture persistence. Pasture diversity alongside defoliation management have shown benefits (e.g., increase herbage production) to pastoral systems (Chapter 6); however, defoliation strategies to enhance soil exploration by deep root species in a mixture needs to be addressed. Such defoliation strategies would allow the pasture to harvest water from deeper soil layers and, thus, favour pasture resilience and persistence (Volaire et al., 2014). Therefore, a defoliation decision approach based on improved below-ground growth could be part of pasture management practices. In addition, whether this defoliation criterion aligns with current defoliation knowledge based on leaf growth needs to be addressed.

Roots are a key element in pastures as their morphology and biomass determines species competition and persistence in the community (Ravenek et al., 2016). There is a large fluctuation in root biomass production throughout the year, with spring being the more productive period. Adventitious roots are produced in early spring and the increase in root growth by elongation is displayed during late spring, and afterwards the production declines during summer. Adventitious root production is resumed in autumn (Garwood, 1967). Parsons (1988) showed that seasonal production of shoots and roots are similar, which indicates that both tissues are dependent on carbon assimilation rates throughout the year. It was assessed that 15% – 20% of assimilates are partitioned towards root growth in grasses (Irving, 2015). However, allocation of assimilates between above and below ground organs drastically changes between functional groups and even within a functional group (Korner, 1991; Poorter et al., 2012), and with change in soil fertility (Saggar et al., 1997).

Recently, Robin et al. (2021) showed root turnover in perennial ryegrass, was dependent on leaf formation and differed between spring and autumn. Robin et al. (2021) observed in spring, tillers of *L. perenne* grown hydroponically produced larger root (higher length and area) and greater fine root volume than autumn tillers. However, autumn tillers produced a larger number and heavier leaves, and a greater number of root bearing phytomers (Robin et al., 2021). This meant that the plant shifted carbon allocation to roots, allocating more carbon towards old root development in spring probably to promote soil exploration before summer (there is a cessation of root growth in summer) as part of a seasonal specialisation of roots. Spring roots explore the soil at depth to supply water during summer; whereas in autumn the root number increases, based on the roots located near the surface (i.e., more phytomer bearing root, but shorter root axis than spring plants) to avoid winter waterlogged of deeper soil layers (Matthew et al., 2016). The appearance of the roots is synchronised with leaf turn over and is a species-specific process (Yang et al., 1998). However, root elongation and final biomass are environment and defoliation regime dependent, as was shown in Chapter 4 (García-Favre et al., 2021b), and carbon allocation to below ground can also change with timing of plant development defoliation (Ilmarinen et al., 2005).

The decrease in defoliation frequency positively affected root production and root elongation in the plant (García-Favre et al., 2021a; García-Favre et al., 2021b), and root biomass content at deep soil layers (Chapter 6). This is closely related with root turnover dynamic (Matthew et al., 2016) and makes defoliation frequency an important tool to increase pastures sustainability improving its resilience and persistence.

Apart from the defoliation frequency effect, root growth can vary between different types of species (fast and slow growing species) or even between similar species differing in functional traits. The growth of fast and slow growing species differs in the allocation of assimilates to shoot and root (increasing root:shoot ratio in slow growing species), which is mainly correlated with soil nutrient pools in the habitat of origin (Poorter & Remkes, 1990). Although *L. perenne* and *B. valdivianus* have similar herbage mass production in monocultures (Chapter 6; Calvache et al. (2020)) and similar soil nutrient requirements (López et al., 1997), they displayed differences in root production at deep layers (Chapter 6). This illustrates that probably small differences in assimilates partition could led to diversification in functional traits between fast growing species. Thus, in the mixture of both species, *L. perenne* contributed predominantly to herbage mass production in near-optimal environmental conditions, while *B. valdivianus* supplied herbage mass production, with a stable tiller population, during the periods of low soil water availability, potentially improving pasture persistence (Chapter 6).

Therefore, the differences in growth between *L. perenne* and *B. valdivianus* determined in the current research can be utilised to seek a defoliation strategy to increase root production of *B. valdivianus* during late spring and thus to increase the summer pasture growth, and persistence of the mixture. Whereas during the time of the year in which species exhibit low root production (winter, autumn, and early spring), the defoliation frequency could shift to a faster one, compared to that performed from late spring through summer. For example, during autumn the frequency of defoliation can be based on *L. perenne* optimal leaf regrowth stage, increasing leaf production, and pasture quality without penalties to pasture persistence. Thus, flexible grazing management throughout the year could contribute to enhanced functional traits of species. However, this asseveration, which constitutes a new hypothesis, needs to be further addressed by studying root dynamics throughout the year and under different environmental conditions and defoliation frequencies.

7.3 TOWARDS INCREASED UTILISATION OF FUNCTIONAL PERENNIAL GRASSES AND IMPLICATIONS FOR ANIMAL PRODUCTION IN ARTIFICIAL PASTURES

Natural grasslands are complex systems that host many different vascular species, which have successfully persisted for hundreds of years under disturbances, such as fire and herbivory (also species succession depends on those disturbance regimes) (Jaurena et al., 2021; Nerlekar & Veldman, 2020). Thus, attempting to mimic natural grassland in a simplistic and less complex may be propitious. When devising a mixed pasture, it is possible to take advantage of the growth of each selected species if different responses to biotic and abiotic factors across species with contrasting functional groups are produced (Cruz et al., 2019). A well-known example of this asseveration is the performance of naturalised pastures in the South of Chile (similar climate region to New Zealand). There, naturalised pastures are composed of perennial and biennial grasses (mainly: *B. valdivianus*, *L. perenne*, *Dactylis glomerata* L., *Holcus lanatus* L, *Trifolium repens* L.), which under management improvements (strategic mineral fertilisation) reached higher annual herbage mass accumulation and also had higher species complementarity (e.g. they capture more water from deep soil layers) than an intensively managed binary mixture pasture (*L. perenne* and *T. repens*) (Descalzi et al., 2020; Ordóñez et al., 2018).

Chapter 6 showed how the addition of a different functional species increased pasture production. Therefore, taking into account the functioning of natural and naturalised pastures in other regions of the world, it is possible that increasing the number of grass species (beyond two species) belonging to different functional groups, or that differ in specific traits, into mixed pastures in New Zealand pastoral system could increase pasture production, persistence and resilience.

However, animal production such as milk, meat or wool under these systems needs to be evaluated. In Chapter 5, animal preference for *L. perenne* and *B. valdivianus* was compared under different seasons. The partial preference that ewes showed for *L. perenne* aligns with its higher quality and morphological characteristics (Chapter 5). This agrees with pasture quality results in Chapter 6, with *B. valdivianus* monocultures showing the lowest nutritive values. It is interesting to highlight that herbage nutritive values of *B. valdivianus* was negatively affected by low defoliation frequency, which suggests there is a compromise between sustaining high root production or high nutritive values in the herbage (Chapter 6). However, the mixture showed high nutritive values under both defoliation frequencies (suitable for supporting high animal production [Freer (2007)]) and root biomass production at depth equal to that of *B. valdivianus* monoculture, evidencing again the importance of mixed species pastures.

The aforementioned partial preference might be different when both species are sharing space and competing for resources; and probably the diet choice by animals when grazing mixed pastures with grasses of different functional groups (when mixing more than two species) could be more complex (Soder et al., 2007), and show a high dependence on seasonal growth among them and grazing intensity. Understanding these processes is important to comprehend species succession within pastures and strengthen the pathway to a sustainable pasture production system, that is more holistic and less demanding of external inputs.

7.4 PRACTICAL IMPLICATIONS

In New Zealand farming systems, there is a proportion of free draining soils (e.g., soils made of loess and river-deposited alluvium) with high soil density, low available soil water content, and a high constraint for herbage growth during dry periods. Only species adapted to these growth limiting conditions can persist and produce adequately. For instance, the coastal and lowland area of western Manawatu-Wanganui region in New Zealand (mean rainfall of 900 mm per year, with frequent dry summers) could take advantage of these species, which could support higher summer production, better recovery after a drought, less weed invasion and higher persistence of mixed pastures compared with the traditional simple binary sown mixture of *L. perenne* and *T. repens*.

Bromus valdivianus showed promising results when sown with *L. perenne* as mixed pasture, due to their high annual herbage mass production (i.e., 15% higher than the monocultures). In addition, the sustainment of the tiller population of *B. valdivianus* over the summer when *L. perenne* decreased its tiller number, due to the low soil water availability, and the greater root biomass at deep soil layers of the former, is expected to result in greater pasture persistence.

Therefore, *B. valdivianus* should be defoliated at the 3.5–4.0 leaf regrowth stage, which means two residual leaves, two full elongated leaves, two expanding leaves and one appearing leaf. However, determining grazing time by looking at the leaf regrowth stage of *L. perenne* (defoliation occurring at 3.0 leaf regrowth stage) is easier determine in the field than it is for *B. valdivianus*. This implies that *B. valdivianus* will be in its optimal leaf regrowth stage, being between 0.4 and 0.6 LS greater than *L. perenne*. In the spring the defoliation frequency could increase (to 2.0 – 2.5 leaf regrowth stage of *L. perenne*), as the high pasture growth rate causes the interception of 95% of the incident light earlier in the development stage of the species. In addition, this is also helpful to control seedhead development and pasture quality of the mixture during spring. However, in late spring, the defoliation frequency should return to the optimal for both species based on leaf regrowth stage and may even be decreased (just over 3.0 in *L. perenne*) to assure higher soil exploration before the start of dry period.

7.4 MAIN CONCLUSIONS

Bromus valdivianus defoliation frequency should be close to the 3.5–4.0 leaf regrowth stage to ensure high herbage mass production and to develop a root system to explore the soil at depth. The mixture of *B. valdivianus* and *L. perenne*, presented similar annual accumulated herbage mass and botanical composition between defoliation frequencies based on *B. valdivianus* or *L. perenne* leaf regrowth stage. This demonstrated that there is a window of opportunity for defoliation of the mixture, as defoliation criteria could be based on the optimal leaf regrowth stage of either species. However, to ensure a higher root biomass at deep soil layers, which could help to sustain a higher pasture resilience and persistence, defoliation frequency should be based on optimal *B. valdivianus* leaf regrowth stage, which has no penalties on the herbage nutritive value of the mixture. *Lolium perenne* dominated in the herbage mass composition in the mixture due to its higher competitive strategy, but its growth and tiller population diminished during low soil water availabilities. Whereas *B. valdivianus* sustained a steady tiller population during the dry period, assuring a better recovery of the mixture afterwards. Therefore, both species not only competed for resources, but complemented each other through a growth asynchrony throughout the year.

Bromus valdivianus as part of mixed pastures could alleviate the effect of low rainfall summers in New Zealand farming systems in the event of climate change (mainly in the eastern country). Animal production could be sustained at high rates as the species showed good overall nutritive values, but this assertion needs to be further addressed.

Every ecosystem is unique, which means it is important to evaluate which species can positively drive ecological function. Thus, a programme of research should be undertaken, i.e., how a grass species needs to be managed in terms of defoliation (to allow functional traits to occur) and how it competes for resources with the main species found in that ecosystem. In the present thesis, it was proved that *B. valdivianus* could agronomically benefit some New Zealand farming systems where soil type suited *B. valdivianus*.

7.5 REFERENCES

Allen, V. G., Batello, C., Berretta, E., Hodgson, J., Kothmann, M., Li, X., . . . Peeters, A. (2011). An international terminology for grazing lands and grazing animals. *Grass and Forage Science, 66*(1), 2-28.

Calvache, I., Balocchi, O., Alonso, M., Keim, J. P., & López, F. I. (2020). Thermal time as a parameter to determine optimal defoliation frequency of perennial ryegrass (*Lolium perenne* L.) and pasture brome (*Bromus valdivianus* Phil.). *Agronomy, 10*(5). https://doi.org/10.3390/agronomy10050620

Chapman, D. (2016). Using ecophysiology to improve farm efficiency: application in temperate dairy grazing systems. *Agriculture, 6*(2).

Clark, D. (2011). Changes in pastoral farming practices and pasture persistence-a review. *NZGA: Research and Practice Series, 15*, 7-13.

Cruz, P., Lezana, L., Durante, M., Jaurena, M., Figari, M., Oliveira, L. B. d., . . . Quadros, F. L. (2019). A functional classification of 63 common Poaceae in the “Campos” grasslands of South America. *Ecología Austral, 29*(2), 239-248.

Descalzi, C. A., López, I. F., Kemp, P. D., Dörner, J., & Ordóñez, I. (2020). Pasture restoration improvement methods for temperate degraded pastures and consequences of the climatic seasonality on soil–pasture complex. J*ournal of Agronomy and Crop Science, 206*(1), 130-147.

Donaghy, D., Bryant, R., Cranston, L., Egan, M., Griffiths, W., Kay, J., . . . Tozer, K. (2021). Will current rotational grazing management recommendations suit future intensive pastoral systems? NZGA: *Research and Practice Series, 17*, 205-222.

Donaghy, D. J., & Fulkerson, W. J. (1998). Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and Forage Science, 53*, 211-218.

Donaghy, D. J., Turner, L. R., & Adamczewski, K. A. (2008). Effect of defoliation management on water-soluble carbohydrate energy reserves, dry matter yields, and herbage quality of tall fescue. *Agronomy Journal, 100*(1), 122-127.

Freer, M. (Ed.). (2007). *Nutrient requirements of domesticated ruminants*. CSIRO publishing.

Fulkerson, W. J., & Donaghy, D. J. (2001). Plant-soluble carbohydrate reserves and senescence —key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental agriculture, 41*, 261-275.

García-Favre, J., López, I. F., Cranston, L. M., Donaghy, D. J., & Kemp, P. D. (2021a). The growth response of pasture brome (*Bromus valdivianus* Phil.) to defoliation frequency under two soil-water restriction levels. *Agronomy, 11*(2), 300.

García-Favre, J., Zhang, Y., López, I. F., Donaghy, D. J., Cranston, L. M., & Kemp, P. D. (2021b). Decreasing defoliation frequency enhances *Bromus valdivianus* Phil. growth under low soil water levels and interspecific competition. *Agronomy, 11*(7), 1333.

Garwood, E. (1967). Seasonal variation in appearance and growth of grass roots. *Grass and Forage Science, 2*2(2), 121-130.

Gastal, F., & Lemaire, G. (2015). Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: review of the underlying ecophysiological processes. *Agriculture, 5*(4), 1146-1171.

Grime, J. P., Hodgson, J. G., & Hunt, R. (2014). *Comparative plant ecology: a functional approach to common British species*. Springer.

Hofer, D., Suter, M., Haughey, E., Finn, J. A., Hoekstra, N. J., Buchmann, N., . . . Bennett, J. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology, 53*(4), 1023-1034.

Husse, S., Huguenin-Elie, O., Buchmann, N., & Lüscher, A. (2016). Larger yields of mixtures than monocultures of cultivated grassland species match with asynchrony in shoot growth among species but not with increased light interception. *Field Crops Research, 194,* 1-11.

Ilmarinen, K., Mikola, J., Nieminen, M., & Vestberg, M. (2005). Does plant growth phase determine the response of plants and soil organisms to defoliation? *Soil Biology and Biochemistry, 37*(3), 433-443.

Irving, L. J. (2015). Carbon assimilation, biomass partitioning and productivity in grasses. *Agriculture, 5*(4), 1116-1134.

Jaurena, M., Durante, M., Devincenzi, T., Savian, J. V., Bendersky, D., Moojen, F. G., . . . Pizzio, R. (2021). Native Grasslands at the Core: A New Paradigm of Intensification for the Campos of Southern South America to Increase Economic and Environmental Sustainability. *Frontiers in Sustainable Food Systems, 5*, 11.

Johnson, K. H., Vogt, K. A., Clark, H. J., Schmitz, O. J., & Vogt, D. J. (1996). Biodiversity and the productivity and stability of ecosystems. *Trends in Ecology & Evolution, 11*(9), 372-377.

Korner, C. (1991). Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. *Functional ecology*, 162-173.

Lemaire, G., Da Silva, S. C., Agnusdei, M., Wade, M., & Hodgson, J. (2009). Interactions between leaf lifespan and defoliation frequency in temperate and tropical pastures: a review. *Grass and Forage Science, 64*(4), 341-353.

López, I., Balocchi, O., Lailhacar, P., & Oyarzun, C. (1997). Characterization of the growing sites of six native and naturalized pasture species in the Dominio Humedo of Chile. *Agro Sur (Chile), 25*(1), 62-80

Matthew, C., Mackay, A. D., & Robin, A. H. K. (2016). Do phytomer turnover models of plant morphology describe perennial ryegrass root data from field swards? *Agriculture*, *6*(3), 28.

McNaughton, S. J. (1977). Diversity and stability of ecological communities: a comment on the role of empiricism in ecology. *The American Naturalist, 111*(979), 515-525.

Nerlekar, A. N., & Veldman, J. W. (2020). High plant diversity and slow assembly of old-growth grasslands. *Proceedings of the National Academy of Sciences, 117*(31), 18550-18556.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology, 46*(3), 683-691.

Ordóñez, I., López, I. F., Kemp, P. D., Descalzi, C. A., Horn, R., Zúñiga, F., . . . Dörner, J. (2018). Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. *Soil and Tillage Research, 178*, 55-64.

Parsons, A. J. (1988). The effects of season and management on the growth of grass swards. In *The grass crop* (pp. 129-177). Springer, Dordrecht.

Parsons, A., Johnson, I., & Harvey, A. (1988). Use of a model to optimize the interaction between frequency and severity of intermittent defoliation and to provide a fundamental comparison of the continuous and intermittent defoliation of grass. *Grass and Forage Science, 43*(1), 49-59.

Pembleton, K. G., Tozer, K. N., Edwards, G. R., Jacobs, J. L., & Turner, L. R. (2015). Simple versus diverse pastures: opportunities and challenges in dairy systems. *Animal Production Science, 55*(7), 893-901.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *The New Phytologist, 193*(1), 30-50.

Poorter, H., & Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia, 83*(4), 553-559.

Ravenek, J. M., Mommer, L., Visser, E. J., van Ruijven, J., van der Paauw, J. W., Smit-Tiekstra, A., . . . de Kroon, H. (2016). Linking root traits and competitive success in grassland species. *Plant and Soil, 407*(1), 39-53.

Reich, P. B., & Cornelissen, H. (2014). The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology, 102*(2), 275-301.

Robin, A. H. K., Irving, L. J., Crush, J., Schnyder, H., Lattanzi, F. A., & Matthew, C. (2021). Time course of root axis elongation and lateral root formation in perennial ryegrass (*Lolium perenne* L.). P*lants, 10*(8), 1677.

Saggar, S., Hedley, C. A., & Mackay, A. D. (1997). Partitioning and translocation of photosynthetically fixed 14C in grazed hill pastures. *Biology and Fertility of Soils, 25*(2), 152-158.

Soder, K. J., Rook, A. J., Sanderson, M. A., & Goslee, S. C. (2007). Interaction of plant species diversity on grazing behavior and performance of livestock grazing temperate region pastures. *Crop Science, 47*(1), 416-425.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science, 277*(5330), 1300-1302.

Turner, L. R., Donaghy, D. J., Lane, P. A., & Rawnsley, R. P. (2006). Effect of defoliation management, based on leaf stage, on perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii* Kunth.) and cocksfoot (*Dactylis glomerata* L.) under dryland conditions. 1. Regrowth, tillering and water-soluble carbohydrate concentration. *Grass and Forage Science, 61*, 164-174.

Volaire, F., Barkaoui, K., & Norton, M. (2014). Designing resilient and sustainable grasslands for a drier future: Adaptive strategies, functional traits and biotic interactions. European *Journal of Agronomy, 52*, 81-89.

Woodward, S. J. R., Van Oijen, M., Griffiths, W. M., Beukes, P. C., & Chapman, D. F. (2020). Identifying causes of low persistence of perennial ryegrass (*Lolium perenne*) dairy pasture using the Basic Grassland model (BASGRA). *Grass and Forage Science, 75*(1),

Yang, J. Z., Matthew, C., & Rowland, R. (1998). Tiller axis observations for perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*): number of active phytomers, probability of tiller appearance, and frequency of root appearance per phytomer for three cutting heights. *New Zealand Journal of Agricultural Research, 41*(1), 11-17.

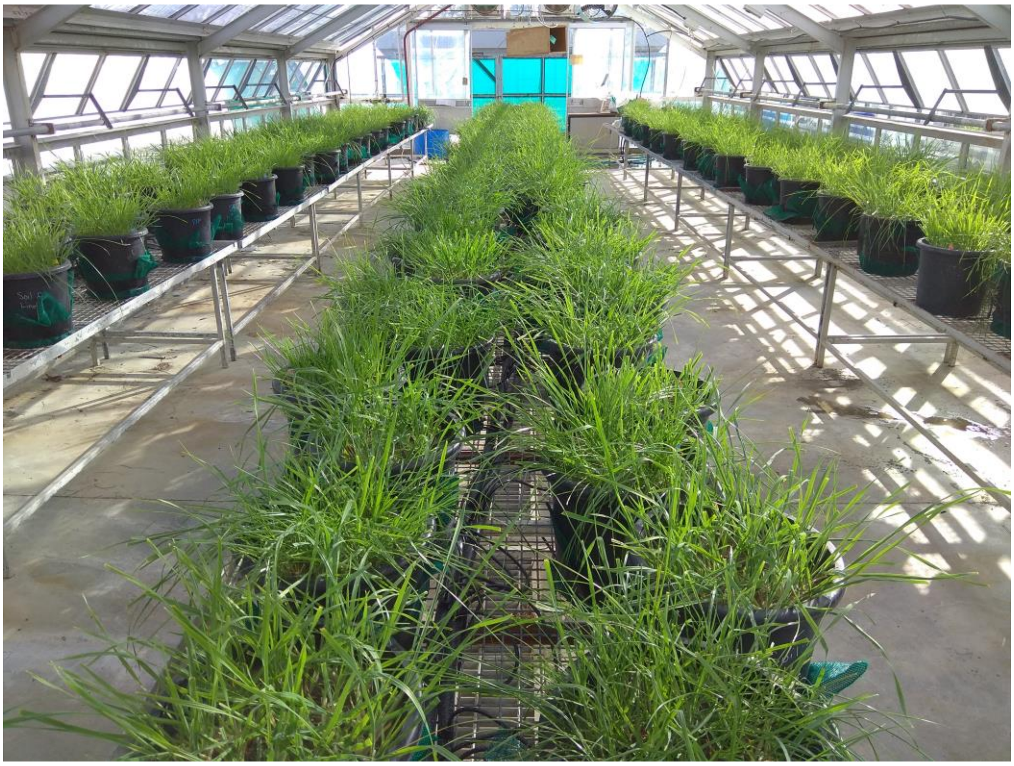
APPENDICES

Appendix 1. Table showing the relationship between leaf regrowth stage and light interception at two defoliation frequencies (Lp LS, based on *L. perenne* [Lp] optimal leaf regrowth stage [LS]; and Bv LS, based on *B. valdivianus* [Bv] leaf regrowth stage) on *L. perenne* and *B. valdivianus* monocultures and the mixture of both species. Within each season, data show average of two years.

	Winter		Spring		Summer		Autumn	
Defoliation frequency	Leaf regrowth stage	Light interception (%)	Leaf regrowth stage	Light interception (%)	Leaf regrowth stage	Light interception (%)	Leaf regrowth stage	Light interception (%)
Lp LS								
L. perenne	2.73 ± 0.08	93.9 ± 0.6	2.57 ± 0.06	95.1 ± 1.3	2.71 ± 0.02	89.8 ± 2.8	2.78 ± 0.06	96.3 ± 0.6
B. valdivianus	3.35 ± 0.08	90.8 ± 1.6	2.78 ± 0.05	94.8 ± 1.3	3.21 ± 0.11	92.2 ± 2.7	3.67 ± 0.08	96.1 ± 06
Mixture (mx)								
L. perenne (mx)	2.71 ± 0.03	93.5 ± 1.0	2.43 ± 0.05	96.7 ± 0.7	2.58 ± 0.03	92.2 ± 2.3	2.88 ± 0.06	95.8 ± 0.4
B. valdivianus (mx)	3.21 ± 0.09		2.90 ± 0.03		3.09 ± 0.03		3.51 ± 0.08	
Bv LS								
L. perenne	3.18 ± 0.05	97.6 ± 0.5	2.57 ± 0.01	96.5 ± 0.5	3.02 ± 0.05	89.8 ± 1.7	3.34 ± 0.03	96.8 ± 0.4
B. valdivianus	3.83 ± 0.05	95.6 ± 0.9	3.05 ± 0.13	93.1 ± 0.9	3.39 ± 0.06	92.0 ± 1.5	3.98 ± 0.08	97.9 ± 0.7
Mixture (mx)								
L. perenne (mx)	3.03 ± 0.05	97.9 ± 0.3	2.60 ± 0.07	97.2 ± 0.4	2.91 ± 0.06	94.9 ± 1.9	2.99 ± 0.09	98.1 ± 0.9
B. valdivianus (mx)	3.98 ± 0.13		3.07 ± 0.08		3.63 ± 0.07		4.04 ± 0.07	

Appendix 2. General view of glasshouses studies (a) and close up to the three defoliation frequency treatments described in Chapter 3 and 4 (b).

a)



b)

Appendix 3. Upper view of the plots of the two years field study described in Chapter 6 (a). Pictures showing a ring in a fixed position to count number of tillers at pasture establishment (b) and at a more developed phase (c). The position was marked with three flathead nails buried in the soil.

a)

b)

c)

Appendix 4. Monoculture of *Bromus valdivianus* close to being ready for grazing and next to it a plot of the same species approaching the post-grazing target.

Appendix 5. The auger used to collect soil and root cores (70 cm depth) at a *Bromus valdivianus* pasture (a). Close up to the roots coming out to the bottom of a core sample collected in a *Bromus valdivianus* and *Lolium perenne* mixture pasture (b).

a)

b)

Appendix 6. Grazing preference trial in winter (a) and in summer during the data collecting period (b). *Lolium perenne* pasture can be identified by a brighter green colour, while *B. valdivianus* by a light green colour and a shorter height.

a)

b)

Appendix 7.

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Javier Horacio Garcia Favre
Name/title of Primary Supervisor:	Ignacio F. López, PhD
Name of Research Output and full reference:	
García-Favre, J., López, I. F., Cranston, L. M., Donaghy, D. J., & Kemp, P. D. (2021). The Growth Response of Pasture Brome (Bromus valdivianus Phil.) to Defoliation Frequency under Two Soil-Water Restriction Levels. Agronomy, 11(2), 300.	
In which Chapter is the Manuscript /Published work:	Chapter 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Conceptualization, methodology, formal analysis, investigation, wrting-original draft preparation	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Javier Horacio García Favre Firmado digitalmente por Javier Horacio García Favre Fecha: 2021.12.21 08:00:30 -03'00'
Date:	21/12/2021
Primary Supervisor's Signature:	Ignacio Lopez Digitally signed by Ignacio Lopez DN: cn=Ignacio Lopez, o=Massey University, ou=Institute of Agriculture and Environment, email=I.F.Lopez@massey.ac.nz, c=NZ Date: 2021.12.21 11:13:29 +13'00'
Date:	21/12/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

GRS Version 4– January 2019

Appendix 8.

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Javier Horacio Garcia Favre
Name/title of Primary Supervisor:	Ignacio F. López, PhD
Name of Research Output and full reference:	
García-Favre, J., Zhang, Y., López, I. F., Donaghy, D. J., Cranston, L. M., & Kemp, P. D. (2021). Decreasing Defoliation Frequency Enhances Bromus valdivianus Phil. Growth under Low Soil Water Levels and Interspecific Competition. Agronomy, 11(7)	
In which Chapter is the Manuscript /Published work:	Chapter 4
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Conceptualization, methodology, formal analysis, investigation, writing-original draft preparation	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Javier Horacio García Favre Firmado digitalmente por Javier Horacio García Favre Fecha: 2021.12.21 08:12:31 -03'00'
Date:	21/12/2021
Primary Supervisor's Signature:	Ignacio Lopez Digitally signed by Ignacio Lopez DN: cn=Ignacio Lopez, o=Massey University, ou=Institute of Agriculture and Environment, email=I.F.Lopez@massey.ac.nz, c=NZ Date: 2021.12.21 11:14:16 +13'00'
Date:	21/12/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)