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**Pursuing pasture tolerance and resilience through species
with different functional traits and soil-plant-water
interactions**

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ABSTRACT

Higher stability, persistence and yield can be achieved through increasing the biodiversity of pasture plants. The combination of species with different functional traits confers niche differentiation (e.g. different root depth). Otherwise species compete for the same resources in the same location and time. In diverse pasture, agricultural needs should overlap between species, enhancing species survival during critical periods. Productive ecosystems with low complexity (low plant functional diversity) show more the negative impacts of climate change, being less stable through the stress periods.

Bromus valdivianus Phil. is regarded as having high potential for grazing systems, due to its high yield and good nutritive quality. It also has high tolerance to periods of soil water restriction, maintaining a higher growth rate during summer in comparison to *Lolium perenne* L. Alongside *L. perenne*, *B. valdivianus* co-dominates permanent perennial pastures in the South of Chile, indicating that it is a good competitor in mixed cool-temperate pastures.

However, key parameters associated with recovery from defoliation, such as watersoluble carbohydrate (WSC) reserves, growth rate, tiller number and persistence, are unknown for *B. valdivianus*. Therefore, the first step in this thesis was to determine these defoliation criteria in relation to similar defoliation criteria of other highly productive species, *L. perenne* and *Plantago lanceolata*, to determine if and when any overlap occurred.

This thesis consisted of three main experiments, which cover the physiological, morphological and competitive traits of *B. valdivianus*. The first experiment was designed to determine a theoretical optimum defoliation interval for *B. valdivianus*, and it was

concluded that defoliation at leaf stage 4 (LS-4) was the optimum defoliation in terms of highest shoot and root growth rates, and accumulation of WSC.

The second experiment was designed to determine the resilience and tolerance between monocultures and mixtures of *B. valdivianus*, *L. perenne* and *P. lanceolata*. All three species were defoliated when *B. valdivianus* reached LS-4, which coincided with approximately 3.5 regrowth leaves/tiller for *L. perenne*, and over 6 leaves/plant for *P. lanceolata*. Measurements included biomass production across critical periods, botanical composition, physiological response against water stress (waterlogging and soil water restriction) and water uptake at different depths, and it was concluded that a more diverse pasture (*B. valdivianus* + *L. perenne* + *P. lanceolata*) maintained higher biomass under soil water restriction and also had a more effective water uptake from the soil profile.

The third experiment was designed to determine the tiller population dynamics, photosynthetic carbon fixation capacity (PCFC) and competitive ability of *B. valdivianus* in relation to *L. perenne*. It was concluded that *L. perenne* was a better competitor than *B. valdivianus*, however, *B. valdivianus* was able to recover its tiller population during a period of soil water restriction and reached a full recovery at the end of the experimental period. Also, a *B. valdivianus* + *L. perenne* mixed pasture had the highest values for PCFC during the waterlogging and soil water restriction periods.

Pasture plants such as *B. valdivianus* and *P. lanceolata* can access water from deeper in the soil than *L. perenne*, having a direct effect on their physiological traits. Water accessibility (root depth) plays a key role in maintaining their photosynthesis, production, and improving their survival, during periods of soil water restriction, relative to *L. perenne*. On the other hand, *L. perenne* tolerate waterlogging and maintain a relatively high growth rates during winter.

Pasture establishment and performance, in a climate with dry and wet seasons and in soils with a high percentage of silt and/or clay (low gas permeability), is related to species tolerance to drought and waterlogging conditions. Thus, increasing species diversity is a good strategy that confers stability to the pastoral ecosystem, especially when global warming has enhanced droughts and unpredictable rain events.

Mixtures of *L. perenne* + *T. repens* + *P. lanceolata* or *L. perenne* + *T. repens* + *B. valdivianus* can reach higher growth rates during water restriction periods, in comparison to *L. perenne* + *T. repens* pastures, along with relatively high growth rates during winter. Therefore, combining species with the aim of complementary resources uptake, and depending on the contribution of each species within the pasture, will change the seasonal herbage growth rate under the stress periods. However, to keep a great contribution of the desirable pasture species it is essential to use a defoliation criterion that allow them to replenish its water soluble carbohydrates, only then, the persistence, survival and yield of the pasture and the desirable species within it will be maximized.

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Glossary of Abbreviations

Abbreviation	Full name/meaning	Unit
AGDD	Accumulated growing degree days	°C day
DM	Dry matter	kg
C	Carbon	
A _n	Net photosynthesis	μmol m ² s ⁻¹
AOS	Active oxygen species	
C _i	Intercellular carbon concentration	μmol mol ⁻¹
CO ₂	Carbon dioxide	
G	Grams	
GDD	Growing degree days	°C day
G _s	Stomatal conductance	mol m ² s ⁻¹
Kg	Kilogram	
kPa	Kilopascal	
LS	Leaf stage	
m ²	Meter square	
Mg	Milligram	
MPa	Megapascal	
PCFC	Photosynthetic carbon fixation capacity	Kg C ha ⁻¹ hr ⁻¹
RCI	Relative competition index	
SEM	Standard error of the mean	
SWC	Soil water content	Vol. %
WSC	Water soluble carbohydrates	mg plant ⁻¹ / mg tiller ⁻¹
WUE	water use efficiency	μmol m ² CO ₂ mol ⁻¹ H ₂ O

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Chapter 1 General introduction and objectives

1.1 Introduction

The current global warming scenario is challenging the traditional management used in many agroecosystems. Extreme periods of drought (Lehner et al., 2006; AghaKouchak et al., 2015), and waterlogging have become more frequent, due to events such as extreme precipitation, increased heat wave frequency (Seneviratne et al., 2012), increased temperature variability making the ambient temperature more unstable (Harrison et al., 2006), and the increasing use of non-renewable water resources for irrigation (Vörösmarty et al., 2005). All these factors interact together with a highly intensive use of the natural resources (Millennium Ecosystem Assessment 2003; Butler and Soskolne, 2013), such that, low diversity agroecosystems are less resistant (Tilman and Downing, 1994; Ulanowicz, 1998; Patsek, 2008) and resilient to environmental stress and disturbances (Ulanowicz, 1998; Tilman, 1999; Patsek, 2008; Isbell et al., 2009; Ulanowicz et al., 2009). To gain ecosystem stability (resistance plus resilience) in a pasture, increasing pasture species diversity has been pointed out as a key factor (Elton, 1956; Tillman, 1999; Loreau et al., 2000; Isbell et al., 2009), together with an increase in functional traits (Hooper and Vitousek, 1997; Hooper and Duke, 2004) and a defoliation criterion according to the species within the pasture ecosystem (Donaghy and Fulkerson, 1998).

Lolium perenne L. and *Trifolium repens* L., are species that concentrate a high proportion of their roots in the shallow soil layers, between 0 and 20 cm (Crush et al., 2005; Ordóñez et al., 2018), because of that, both species are susceptible to water restriction periods (Ordóñez et al., 2018), but show a high tolerance to waterlogging conditions (McFarlane et al., 2003) and share similar colonisation strategies, classified as ruderal - competitor

(Grime et al., 1977). Pasture height has been used as grazing indicator for *L. perenne*, however, neither pasture height nor herbage mass are directly related to phenological maturity, nor are they indicators of water soluble carbohydrate (WSC) reserves that have refilled as plant regrowth occurs after defoliation (Donaghy and Fulkerson, 1997; 1998). It was determined for grasses that leaf stage was a good indicator for when to defoliate, as it directly related to plant phenological development (Donaghy and Fulkerson, 1997, 1998; Turner et al., 2006a,b,c,d), such that, for *Lolium perenne* defoliation at the 2.5 leaf stage encourages regrowth after defoliation and persistence (Donaghy and Fulkerson, 1998). In cool-temperate climates, *L. perenne* is one of the most frequently used grass species, constituting the base of the sown pastures in New Zealand (Amin and Thomas et al., 1996; Kemp et al., 2002).

Plantago lanceolata has a deep root system that allows it to tolerate soil water restriction (Sanderson et al. 2003; Nie et al. 2008). Lee et al. (2015) reported that, for a full water soluble carbohydrates reserves recovery after defoliation, the plant needs to accumulate 532 °C AGDD (accumulated growing degree days).

Bromus valdivianus Phil. is tolerant to soil water restriction (López et al., 2013; Ordóñez et al., 2018), highly productive with high quality forage (López et al., 2013), but its defoliation criterion has not been defined according to the energy reserves-leaf stage interaction.

The presence of species with different root distribution within the same pasture ecosystem, such as shallow-rooted (*L. perenne* and *T. repens*) and deep-rooted species (*P. lanceolata* and *B. valdivianus*) should provide a higher biomass stability in terms of annual pasture herbage mass production and also across seasons. However, the defoliation criterion for the drought tolerant *B. valdivianus* needs to be defined based on

its growth physiology, in which leaf stage should be a good indicator parameter. In a diverse pasture, a common window opportunity between the species for defoliation is a target, as defoliation is tool to encourage species growth.

According to the background provided, the hypothesis of this thesis is that an increase in functional diversity, through combining species tolerant to waterlogging and also soil water restriction, which share a common defoliation window opportunity, enhances species survival after soil water stress, providing a higher stability and biomass production during critical periods than monocultures.

Therefore, the objectives of this thesis are as follows: 1) To determine the defoliation criterion for *B. valdivianus* grass; 2) To determine the botanical composition and herbage mass production of pastures containing *L. perenne*, *T. repens*, *B. valdivianus* and *P. lanceolata* as monocultures and mixtures across seasons; 3) To examine the physiological parameters for *L. perenne*, *B. valdivianus* and *P. lanceolata* as monocultures and as pasture mixtures across seasons; 4) To determine the changes in tiller dynamic/population, under intra- and interspecific competition between *L. perenne* and *B. valdivianus* as monocultures and as pasture mixtures across seasons.

1.2 Thesis structure

Seven chapters composed this thesis. Chapter 1 is the introduction, Chapter 2 provides the concepts and the current knowledge of the effects of diversity on plants and ecosystems.

Three research chapters are included: Chapter 3, determined the defoliation criterion for *B. valdivianus*, Chapter 4 determined whether *B. valdivianus* defoliation criterion determined in Chapter 3 affected the persistence of *B. valdivianus*, *L. perenne* and *P.*

lanceolata, and how the growth dynamics changed over time in monoculture and in mixed swards, with varying soil water content. Chapter 5 determined the tiller dynamics and the outcome of the competition between *L. perenne* and *B. valdivianus* across several seasons and estimated the potential photosynthesis for both species as a function of changes in tiller population.

Chapter 6 discusses the main findings of the thesis and Chapter 7 presents the conclusions of the thesis.

Chapter 2 Literature review

2.1 General concepts behind the diversity-stability theory

Several authors have suggested that ecosystem stability increases with a higher species diversity (Elton, 1958; Tilman and Downing., 1994; Hooper and Vitousek, 1997; Tilman et al., 1997; Tilman, 1999; Loreau et al., 2000; Loreau and Hector 2001; Loreau et al., 2001; Hooper and Dukes, 2004; Tilman et al., 2006; Isbell et al., 2009; Tilman et al., 2014). The ecosystem organisms are responsible for different process (Tilman, 1999) led by their own species traits (Tilman et al., 1999; Hooper and Vitousek, 1997). This range of species traits is a main factor leading to higher stability, higher productivity and better nutrient cycling. Thus, different species traits lead to a greater access to available resources, or in other words, they are able to explore different ecological niches (Hooper and Vitousek, 1997; Loreau and Hector, 2001). Therefore, the complementary resource use (Trenbath, 1974; Hooper, 1998) can happen in a common space (e.g., different root depth and/or traits) and time (e.g. growth asynchrony; Hooper and Vitousek; 1997; Isbell et al., 2009), increasing the harvesting efficiency of the resources (Elton, 1958; Naeem et al., 1996; Tilman, 1999; Tilman et al, 2006; Loreau, 2000; Loreau et al., 2001; Hooper and Dukes, 2004).

Three mechanisms have been recognized as responsible for increasing the ecosystem stability, and therefore, sustainability (Ulanowicz, 1998; Tilman, 1999; Patzek, 2008; Ulanowicz et al., 2009) and they are as follows; portfolio effect (Tilman et al., 1999; Isbell et al., 2009),overyielding (Tilman et al., 2009; Isbell et al., 2009; Hooper and Vitousek, 1997; Hooper and Dukes, 2004) and asynchrony (Loreau et al., 2001; Isbell et al., 2009; Zhang et al., 2018). The portfolio effect, a concept originally created as a probabilistic financial investment theory and thereafter applied in ecology and evolution disciplines,

states that the investment in several portfolios produces more stable returns than a simple portfolio (Schinder et al., 2015). According to this theory, biological properties are scale-dependent, which means that the stability of the properties could change in function with the scale of the observation (Levin, 1992). For example, in plant ecology the stability of a single species is lower than the whole ecosystem stability (Tilman, 1999; Lehman and Tilman, 2000; Isbell et al., 2009). Overyielding acts as a stabilising factor within a plant community biomass (Lehman and Tilman, 2000; Isbell et al., 2009) and it occurs when the community biomass increases together with the biodiversity (Harper, 1977; Tilman et al., 1996; Isbell et al., 2009), the overyielding could be explained by niche partitioning (complementary resource use) and resource facilitation hypotheses (Trenbath, 1974; Hooper, 1998; Hooper and Duke, 2004), caused by the presence of different plants functional groups (Hooper and Vitousek, 1997). Asynchrony is when the plants development occurs at different times of the year, stabilising the biomass production of the ecosystem (Tilman, 1999; Loreau and Hector, 2001; Isbell et al., 2009; Zhang et al., 2018).

2.2 Sustainable production: stability related to sustainability

Ecosystems have been defined as the “basic unit in nature” for which biotic factors are in interaction with abiotic factors (Tansley, 1935). The Millennium Ecosystem Assessment (2003) divided the services delivered by an ecosystem as follows: provisioning (e.g. food), regulating (e.g. soil erosion reduction), culturally enriching (e.g. ancestral links) and supporting (e.g. nutrient cycling; Butler and Soskolne, 2013). Human activity has been indicated as responsible for the abrupt changes in the ecosystems and species within (Soskolne et al., 2008). Butler and Soskolne (2013) reported that the ecosystems services have been changed drastically towards provisioning, leaving aside the services related to

regulating and supporting, both necessary to build sustainable production. But even if one of the causes is agricultural activity, the food, biofuel or medical agents production are necessary for the human wellbeing, especially due to the increasing population (over 7 billion), where natural systems (wild) are unable to provide enough goods to sustain life as agriculture does (Butler and Soskolne, 2013).

To reach a sustainable agriculture it is necessary build ecosystems more stable to environmental constraints (Tilman and Downing, 1994; Tilman, 1999; Isbell et al., 2009). But there are two concepts inside the word “stability”, one of them is the static aspect related to an ecosystem’s continued existence (resistance), and the other is the dynamic aspect, or the capacity of the ecosystem to return to its “original state” (resilience) (Gigon, 1983). Therefore, ecosystem stability means that an ecosystem can maintain and recover its integrity and structure after a disturbance/stress has occurred (Holling, 1973). This concept is essential to build sustainability in productive systems (resilience and resistance).

To maintain high biomass production (high energy outputs), from an ecosystem through time, high energy inputs are required (Odum and Pinkerton, 1955). Odum and Pinkerton (1955) discussed the application of the thermodynamic theory to agricultural systems, which indicates that a maximum input of energy is always less than the greater efficiency possible in agroecosystem. Therefore, goods production using high energy inputs, e.g. irrigation, high fertiliser dose, soil preparation, pesticides helped to build low efficient and unstable agroecosystems. In the same line, Ulanowicz, (1998); Patzek et al., (2008) and Ulanowicz et al. (2009) stated that agroecosystem sustainability is achievable if there is a right proportion between efficiency in biomass production and resilience, and also indicated that sustainable agroecosystems cannot be achieved without biodiversity, and

being disorderly and moderately inefficient. Similar statements have been provided by Tilman (1999); Tilman et al. (2006); Isbell et al (2009), arguing that a higher stability (due to higher biodiversity) is also related to a low stability for a single species (disorderly; portfolio effect). Opposite to the productive systems based on monocultures, which are systems with lack of complexity (no disorder) and with a lack of biodiversity and therefore lower resilience to disturbance/stress (Patzek, 2008). These could be the reasons why an agroecosystem based on just one species is sensitive to any stress/disturbance that affect that species, negatively affecting the whole biomass production (Tilman et al., 1994; Ulanowicz, 1998; Ulanowicz et al., 2009).

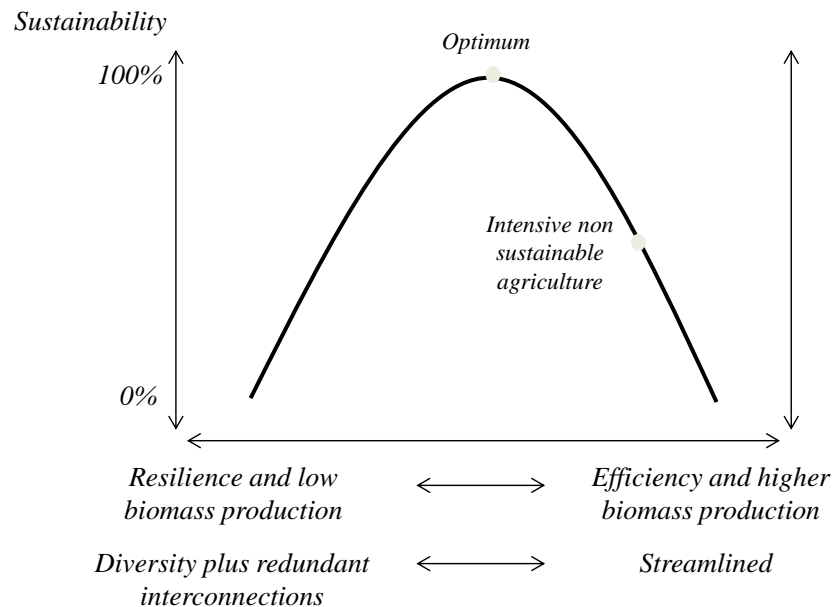


Figure 2.1 Adapted from Patzek et al. (2008) taken from Ulanowicz et al.’s theory. Shows the optimum point of sustainability in comparison to the current agricultural management. Traditional maize crops were taken as an example of intensive non-sustainable agriculture.

Figure 2.1 illustrates that to increase the resilience of agroecosystems it is necessary to sacrifice biomass production, which also allows the agroecosystem to reach sustainability. This indicates that stability and, therefore sustainability, can be achieved

at low diversity but with different species functional groups (functional traits) within the ecosystems (Hooper and Vitousek, 1997).

Increasing ecosystem complexity is not just related to an increase in plant species diversity or species functional diversity, it is related to all the interactions between the different traits of the plants species or biotic factors within the environment or abiotic factors. For example, an increase in soil pore continuity and therefore an increase in gas diffusion in a pasture ecosystem (Peth et al., 2010; 2008) can result from an increase in soil pore continuity at deep soil layers due to deep-rooted species (Uteau et al., 2013). In southern Chile, naturalised pasture with a low biomass production and with high plant diversity were negatively affected by the diminishment of soil mechanical properties due to animal trampling (Zúñiga et al., 2015; Ivelic-Sáez et al., 2015; Ordóñez et al., 2018). Also in southern Chile, in fertilised naturalised pastures with a high biomass production and with a lower plant biodiversity, in comparison to the non-fertilised naturalised pastures, the water uptake between 0-60 cm soil depth was more evenly distributed (Ordóñez et al., 2018). The higher water uptake at deep soil layer indicates a greater effectiveness for water uptake (Blum, 2009) when different root traits are present in the pasture. From these experiments it can be concluded that biodiversity is not enough to reach sustainable production. Both, biotic factors (e.g. plant parameters) and abiotic factors (e.g. soil water dynamics) and their interaction need to be understood to reach to appropriate conclusions related to the ecosystem stability and sustainability.

2.3 The effect of an increase in plant diversity

The research by Elton (1954); Tilman (1999); Loreau, 2000; Loreau et al., 2001 and Isbell et al. (2009) shows that there is a positive correlation between increasing biodiversity and pasture survival, persistence, resilience and production during and after abiotic stress or

disturbance (e.g. water restriction). Plants species drive a variety of ecological processes by altering the ecosystem according to their own traits (Tilman, 1999), such as nitrogen fixation (e.g. legumes) or alteration of soil porosity due to deep-rooted species (e.g. *B. valdivianus* and *P. lanceolata*). Therefore, plant species can generate changes in an ecosystem by modifying soil nutrient status (Fornara and Tilman 2009), soil structure (Haynes and Beare, 1997; Kabir and Koide, 2000; Moreno-Espindola et al., 2007) or soil water dynamics (Gan et al., 2012; Ordóñez et al., 2018). Along the same line, Petchey et al. (2004) indicated that a more diverse pasture raises the probability of an increase in traits within the pasture, building a more complex ecosystem, with a stable functioning against the impact of environmental constraints. According to the role of each species involved, the increase in functional diversity in a productive system may help it to reach greater sustainability and stability over time.

Currently, the use of pastures based on one grass species and one legume species has become the most common manner to produce pastures in cultivated soils for grazing production systems (Kemp et al., 2002). The research realised until now has indicated that a pasture with several species can enhance the resource acquisition, such as, a higher presence of soil nitrogen, given by the legumes (Schwinning and Parsons, 1996; Fornara and Tilman 2009), high root biomass and root activity (Tilman et al., 1996), positive effect of plant nutrient concentration to soil nutrient availability (Fornara and Tilman 2009), water lifting generated by deep root species (Richards and Coldwell, 1987; Caldwell and Richards, 1989; Skinner et al., 2008).

2.3.1 The effect on plant parameters

Long-term experiments have shown that pasture biomass and its stability are strongly related to an increase in plant diversity, where the stability was assessed in terms of dry

mass production, across several years (Tilman, 1999; Loreau, 2000; Tilman et al., 2006; Isbell et al., 2009). But it has been noticed, that contrary to the stability of the ecosystem, survival of individual species declined with higher plant diversity, but overall, this led to greater stability (Portfolio effect; Tilman et al., 1999; Isbell et al., 2009). Moreover, overyielding refers to herbage mass production that exceeds the expectations based on the most productive monoculture (Hooper and Duke, 2004), which results from two mechanisms, complementary resources use and facilitation (Trenbath 1974; Hooper and Duke, 2004). The complementary resources use, could happen in a different space (e.g. root depth and leaf canopy architecture), time (e.g. phenology) or resources type (Hooper and Duke, 2004), and the facilitation is related to species that can deliver limited resources, e.g. legumes ability to fix atmospheric nitrogen (Schwinning and Parsons, 1996; Hopper and Duke, 2004). In the treatments without legumes, overyielding could occur by the different root depth or phenology of the species within. Therefore, in a cool temperate climate in which water is a limited resource during the summer period, species with different root depths can lead to pasture ecosystem overyielding (Hooper and Duke, 2004).

Mueller et al. (2013) in a study of 12 years in Minnesota, USA, with pastures that range from 1 to 16 species per treatment, showed that high diverse pastures had a greater increase of foliage and root biomass in relation to monoculture pastures. The sampling for the aboveground and root biomass evaluations were done during August 2006 (12 years after the pasture establishment). The above ground production increased from 100 g m² (1 species treatment) to 375 g m² (16 species treatment), and the root biomass from 500 g m² (1 species treatments) to over 1500 g m² (16 species treatment). These findings were related to the presence of species with different functional traits in the more diverse pasture, such as C4 species and legumes.

Liu et al. (2016) in Inner Mongolia, China, determined the effect of a monoculture and mixed of two (*Medicago sativa* L. + *Bromus inermis* Leyss.) and three (*M. sativa*, *Bromus inermis* and *Elymus nutans* Griseb.) pasture species on the production above and below ground. The above ground mass production was greater for the mixture of *M. sativa* + *B. inermis* (over 350 g m²). The below ground mass production was greater for *M. sativa* (over 650 g m²) followed by the mixture *M. sativa* + *B. inermis* (over 550 g m²). The root-shoot ratio was 6.5 times greater for *M. sativa* monoculture, indicating a superior production below ground than above ground. On the other hand, when *M. sativa* + *B. inermis* mixture was compared, the below ground mass production was 1.5 times greater than above ground mass production. Also, they showed differences in net photosynthesis, where the *Bromus inermis* in the mixture had greater photosynthetic rate in comparison to *Bromus inermis* in the monoculture, which makes sense due to the N fixation by the *M. sativa*. Nevertheless, independent of how much root mass *M. sativa* monoculture presented, the transpiration rate and water use efficiency were the same.

Hooper and Vitousek (1997) indicated that a pasture system increases its production with a greater functional group number due to the complementarity of the species. The functional group composition in a pasture is more relevant than the species richness by itself. This suggests that the combination of the functional properties' controls yield quantity. It has been suggested that in a pasture, a maximum of 4 functional groups (Figure 2.2, adapted from Hooper and Vitousek (1997) maximises above ground production.

In New Zealand, little research has been performed on the mechanisms that regulate production as function of diversity. In a study with *L. perenne*, *T. repens*, *Plantago lanceolata* L. and *Trifolium pratense* L., with different sowing rate under irrigation (Black

et al. (2017)), it was estimated that a greater yield can be achieved when 3 species are established, *L. perenne*, *P. lanceolata* and *T. pratense*, all of them with different functional traits. This result was supported by *T. pratense* N fixation and the possible niche partitioning involved.

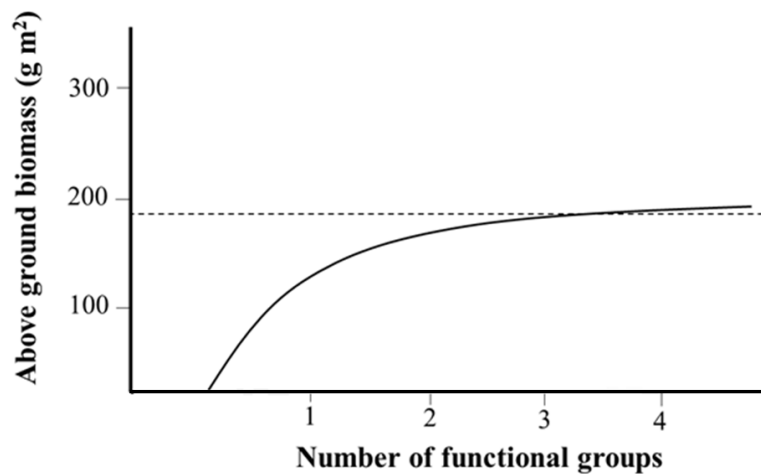


Figure 2.2 Adapted from Hooper and Vitousek (1997). Simplified figure of the number of functional groups required to reach a greater above ground biomass production.

Woodward et al (2013) evaluated 2 types of pastures; 1) *L. perenne* with *T. repens* and 2) *Bromus willdenowii* Kunth. + *Cichorium intybus* L. + *P. lanceolata* + *M. sativa*. No statistical differences were assessed, producing in three years an average yield of 14.7 and 15.3 ton DM ha⁻¹ year⁻¹, respectively.

Goh and Bruce (2005) evaluated the following pastures: 1) *L. perenne* + *T. repens*; 2) *Festuca arundinacea* Schreber + *Dactylis glomerata* L. + *Bromus stamineus* E. Desv. + *Phleum pratense* L. + *Phalaris aquatica* L. + *T. pratense* + *Trifolium ambiguum* M. B. + *T. repens* + *M. sativa* + *Hedysarum coronarium* L. + *C. intybus* + *P. lanceolata* +

Sanguisorba minor Scop. + *Achillea millefolium* L.; 3) *M. sativa* + *F. arundinacea* + *B. stamineus* + *P. pratense* + *C. intybus* + *P. lanceolata* + *S. minor* + *A. millefolium*; 4) *T. pratense* + *T. repens* + *F. arundinacea* + *B. stamineus* + *C. intybus* + *P. lanceolata* + *S. minor* + *A. millefolium*. The non-irrigated pastures showed no significantly statistical differences for the annual yield, between 6.0 and 7.2 ton ha⁻¹ year⁻¹. Nevertheless, the pastures presented different biomass production distribution of across the year.

One of the complications in determining the root mass in the field is that live and dead roots are included. Skinner (2008) indicated that *C. intybus* L. + legume + grass mixture, under water stress, had higher production in comparison with the legume + grass mixture, nevertheless, the mixture with a higher root mass below 70 cm was the mixture legume + grass. Regarding the soil water content at 25, 50 and 75 cm, the authors did not present any differences between the two and three species mixtures, reporting the same water uptake. From this it may be assumed; 1) different water use efficiency between the mixes and/or photosynthesis rate; 2) no strong relationship between the total root mass with the water uptake; 3) no differences on quantity of functional roots between the mixtures. The quantity of roots was not reflected in the water uptake indicating that a greater part of root mass was not functional, and the three-mixture treatment had less quantity of roots (below 70 cm), but may have had a higher proportion of live roots. Therefore, one of the complications in determining the root mass is that it often includes both live and dead roots. From this, it can be concluded that the root mass by itself is not a good indicator for water uptake and/or the quantity of functional roots.

Ordóñez et al. (2018) evaluated the SWC at different depths and showed SWC to be a parameter sensitive enough to determine differences in root distribution in the soil profile. Therefore, a good methodology to determine root functionality, indirectly, is the

evaluation the soil water content (Skinner et al., 2008; Neal et al., 2012; Ordóñez et al., 2018).

2.3.2 The effect on water parameters

Soil water content (SWC) is a function of water infiltration, precipitation, evaporation and the water uptake by plants (Simunek et al., 2003). Plant water uptake is proportional to plant transpiration (Skinner 2008; Blum, 2009), being directly related to the functional roots of the species plus root distribution in the soil (Neal, et al., 2012; Ordóñez et al., 2018). Neal et al. (2012) indicated that *L. perenne* had less water uptake in deep soil layers compared to *B. willdenowii*, *F. arundinacea*, *P. lanceolata* and *P. aquatic*. *Lolium perenne* showed a soil water deficit around 13 -19 mm between 1 – 1.5 m soil depth. At the same depth soil layer, the other grasses and herbs showed a maximum value between 34 - 49 mm of water deficit. Thus, *L. perenne* extracted less water than the other species tested but it was able to extract water up to 150 cm soil depth. Therefore, *L. perenne* has the capacity to reach deep soil layers, nevertheless, with low capacity of soil water extraction, probably due to few functional roots are able to reach that depth.

The relationship between diversity and the soil water dynamics was demonstrated by a highly productive pasture with high pasture species diversity having a greater water uptake at 60 cm soil depth compared to a pasture dominated by *L. perenne*, especially over the periods of water restriction (summer; Ordóñez et al., 2018). Nevertheless, *L. perenne* enhanced water uptake in the first 10 cm of soil, which means that the quantity of functional roots developed in the first 10 cm by *L. perenne* was significantly higher (Ordóñez et al., 2018) and/or presented, as a species, better physiological adaptations for water restriction (e.g. higher osmotic adjustments), allowing it to increase its water uptake at high levels of soil matric potential (Chimenti et al., 2006). Therefore, the pasture soil

water dynamics would differ due to the botanical composition of species roots traits and physiological traits.

An evenly water uptake through the soil profile was observed due to a mixing shallow-rooted species (species with a lower quantity of roots reaching the deep soil layers), and deep-rooted species (species with a high quantity of roots reaching the deep soil layers) (Ordóñez et al., 2018). Also, water redistribution mechanisms need to be considered, such as water lifting (Richard and Caldwell, 1987; Caldwell and Richards, 1989; Caldwell et al., 1998; Skinner, 2008) and the soil pore continuity, both of which may play an important role related to the water availability in the shallow soil layer.

Similarly, Skinner et al. (2004) undertook a pasture study that included some mixed pastures with and without *Cichorium intybus*. The results indicated that a shallow-rooted species (*T. repens*) had a higher leaf water potential compared to *T. repens* in pastures without *Cichorium intybus*, indicating that chicory was aiding the survival of the shallow-rooted species, possibly by hydraulic lifting generated by *Cichorium intybus*, a deep-rooted species (Caldwell and Richards 1989; Caldwell et al., 1998).

Water lifting, in theory, works when the soil water potential is more negative than the root xylem water potential. Once this happens, there must be a movement of water from the root to the soil, using the root as a bridge between the bottom of the soil and the top (Caldwell et al., 1991). Richards and Caldwell (1987) observed, in an experiment with *Artemisa tridentata* Nutt, that the soil water potential at 35, 50 and 80 cm of soil depth had the same daily variation, with increases and decreases, especially during the dry days. This variation in soil water potential, stopped when there was an important addition of water, like rain events.

Skinner (2008) evaluated the soil water potential and the soil temperature of the superficial soil layer in plots under environmental controlled conditions (glasshouse). The water potential and the temperature of the superficial soil layer showed the same tendency as that reported by Richard and Caldwell (1987), which consisted of a diurnal fluctuation in the soil water at 25 cm soil depth, and the water lifting occurring at night-time. In addition, there was no significant relationship between the changes in the soil matric potential and the temperature ($r = -0.03$). Therefore, the increase in water potential at night time had no relationship with the soil temperature, and was probably due to the water lifting mechanism, also called hydraulic lift, discarding the significant effect of the temperature over the soil matric potential sensor measurements, as was previously indicated by Wiebe and Brown (1979). This mechanism has been also observed in *L. multiflorum* (Meunier et al., 2017), in legumes (Pang et al., 2013), in oak trees (Zapater et al., 2011) and in senescent tissue of grasses (Leffler et al., 2004).

2.4 Perennial species growth mechanisms

To understand how to reach a more resilient and resistant ecosystems, the growth and development of the different plant's species need to be understood. Plant growth process is stimulated or repressed by the environmental conditions or abiotic factors, such as, temperature, light, water and nutrient availability (Bullock et al., 1994). In the vegetative stage, a grass grows due to the cell division from the meristematic tissue (Dahl, 1995) located at ground level (Glémin and Bataillon, 2009), whereas during the reproductive stage the apical meristem goes up the stem until the inflorescence structure develops (Dahl, 1995). The change from the vegetative stage to the reproductive stage is dominated by temperature and photoperiod (Langer, 1972; Dahl, 1995; Gastal and Durand, 2000).

In the reproductive stage the vegetative development (tiller production and leaf appearance and growth) stops (Langer 1972; Matthew, 2000).

In the vegetative stage the leaf growth starts from inside the sheaths, from the meristematic cells called phytomeres, and once the new growing leaf is exposed to light the photosynthesis starts (Langer, 1972; Dahl, 1995). A new leaf growing immediately after defoliation utilises the energy from the reserves of water soluble carbohydrates (WSC) located in the stubble (Fulkerson and Slack, 1994a; Donaghy and Fulkerson, 1997; Turner et al, 2007b), roots, rhizomes (Trlica et al., 1977) or stolon (Inostroza et al., 2018), until the requirement for growing and development can be fulfilled by photosynthesis (Langer, 1972; Fulkerson and Slack, 1994a).

The senescence starts from the leaf tip and develops towards the base (or from the older cells to the youngest ones), and during vegetative development the start of senescence is variable according to the species. For example, *L. perenne* can hold 3 live leaves and when the fourth leaf appears, the older leaf starts to senesce (Donaghy and Fulkerson, 1997,1998; Fulkerson and Donaghy, 2001), whereas *Dactylis glomerata* L. can hold 4 live leaves (Turner et al., 2006d). The consequences of the leaf aging are highly correlated with an increase in active oxygen species (AOS), compounds that generate the leaf cell peroxidation (Cakmak and Horst, 1991; Singh Gill and Tuteja, 2010), decreasing the leaf photosynthetic capacity (Langer, 1972; Xu et al. 2011).

Tillers are considered grass growth units (Matthew et al., 1998), with the shoots derived from a vertical growth of an axillary bud (Dahl, 1995). A fully developed tiller is potentially an independent unit with leaves, stem and roots (Dahl and Hyder 1977; Dahl 1995). All daughter tillers are dependent on the main tiller or shoot, for WSC or energy (Alberda, 1966; Volaire et al., 1995; Donaghy and Fulkerson, 1998), until they grow their

own root system (Dahl, 1995). Tillers maintain their vascular connection with the other tillers even after they have developed their own roots, allowing the flow of water, soil nutrients and photosynthesis assimilates amongst them (Dahl and Hyder 1977; Dahl 1995). Therefore, a depletion of the WSC in all plants defoliated (e.g. grasses and herbs such as *P. lanceolata*) will have a negative effect on its the persistence and survival (Donaghy and Fulkerson, 1998).

2.5 Root growth and development

The root system varies in architecture, expressed as a variation of the distribution of root length in space, the dominance of the main axis, the relative sizes of the members of the root system and the geometry of the branching pattern. These variations in root form, are the result of the genetic-environment interaction (Fitter, 1991).

The roots perform several functions in the lifespan of a plant (e.g. mechanical support) but there are two that are of major importance: the acquisition of soil nutrients and water uptake. Roots also have other functions such as storage of resources (e.g. *P. lanceolata*) and some metabolic process (Fitter, 1991).

In terms of the total production of plants, it has been estimated that approximately 70-85% of the total mass produced for a plant occurs below ground (Caldwell and Richards, 1989). Research using C^{14} showed that around 13 - 25% of the total carbon fixation by photosynthesis is transformed into biomass within ten days of the C absorption, being the variability caused according to the agronomical managements applied, such as defoliation or fertilisation (Wang et al., 2007; Pausch and Kobayakov, 2018). Different carbon allocation patterns can be found for different species functional groups, such as tall or short grasses species (Siebenkäs et al., 2015) and by differences related to transpiration rates and water use efficiency (Poorter and Farquhar, 1994).

According to Matthew et al., (1991) one of the variables that affects *L. perenne*'s root mass is grazing. Therefore, grazing management as a function of the defoliation criterion could affect the root distribution and the resource uptake during periods of stress, such as water uptake during soil water restriction periods, statements that are in line with Wang et al. (2007).

The turnover of the roots likely indicates a relationship between root and tiller appearance rate, with seasonal patterns for both (Matthew et al., 1991). Descalzi (2011) showed that *B. valdivianus* tiller appearance and death were more stable during all the seasons compared to *L. perenne* under intensive grazing (defoliation criterion of 2.5 leaf of *L. perenne*). Therefore, it can be assumed, that the root appearance for *B. valdivianus* may be more constant and stable in time than *L. perenne*'s roots.

Besides the management, type of species and soil water restriction, another climatic-soil condition interaction that highly affects and reduces the quantity of pastures roots is waterlogging, resulting in a loss of root mass in response to the lack of oxygen in the soil (Drew, 1997). For example for *D. glomerata* diminishes from 0.10 (control) to 0.075 g g⁻¹ day⁻¹ (waterlogging) in root mass, showing negative values during the recovery period. *Bromus catharticus* (sin. *B. willdenowii*) also shows a negative recovery after a waterlogging period. On the other hand, species better adapted to this condition, such as *F. arundinacea* and *P. aquatica* had a positive recovery after the waterlogging, showing no statistical differences of root recovery of the control and waterlogging treatments with values around 0.05 and 0.025 g g⁻¹ day⁻¹ in root mass, respectively (Ploschuk et al., 2017).

2.6 Defoliation effect on perennial species

Defoliation increases the meristem activity and a compensatory physiological process (Briske and Richards 1995), due to the removal of photosynthetic tissue (Richards and

Caldwell 1985; Chapman and Lemaire 1993; Briske and Richards 1995). Therefore, the regeneration of the photosynthetic leaf area is essential for the plant and pasture recovery after defoliation (Chapman and Lemaire 1993; Richards, 1993; Langer, 1972; Dahl, 1995; Donaghy and Fulkerson, 1998). This restoration is caused by the cell production, expansion and thereafter differentiation, in the meristem located in the base of the leaf (MacAdam et al. 1989; Schnyder et al. 1990). Research indicated that the leaf elongation rate diminishes due to defoliation, affecting negatively the cell size (Schaufele and Schnyder, 2000), which also had a direct relationship with the water-soluble carbohydrates storage (Lattanzi et al., 2004), as less WSC content, due to the increasing defoliation frequency, could lead to a detrimental effect on persistence and survival due to a depletion of tiller population (Fulkerson and Donaghy, 2001; Donaghy and Fulkerson, 1998; Turner et al., 2006a,b,c,d).

2.6.1 Water soluble carbohydrates storage

Defoliation frequency has a direct effect on the WSC storage (Alberda, 1966; Turner et al., 2006a,b,c,d; Donaghy and Fulkerson, 1998; Lee et al., 2015) in herbs (e.g. *P. lanceolata*) and grasses (e.g. *L. perenne* and *B. valdivianus*). WSC stored is essential for tiller and plant regrowth (Donaghy and Fulkerson, 1997,1998; Turner et al., 2001). Therefore, the defoliation frequency, in relation to the grass and herb vegetative development, will define the content and concentration of the plant WSC stored. If the defoliation frequency is too fast, the plant energy reserves will not be fully restored at the time for the following defoliation, negatively affecting the plant's development, survival and persistence (Volaire et al., 1995; Donaghy and Fulkerson, 1998), and plant survival during water restriction (Volaire et al., 1995) and waterlogging (Ploschuk et al., 2017).

It had been determined that every species has an ideal defoliation frequency according to its energy storage. For example, for *D. glomerata* the WSC content varies from 218 to 1,477 mg plant⁻¹ depending on the defoliation frequency. For *L. perenne* it has been shown that upwards of 160 g WSC kg⁻¹ there was no effect on regrowth (Donaghy and Fulkerson, 1997). For *B. wildenowii* it has an ideal content of 600 mg WSC plant⁻¹ at defoliation (Turner et al, 2007c), therefore the variation due to defoliation frequency and the effect on WSC is evident, and it varies as a function of the plant species (Turner et al., 2006a,b,c,d; Fulkerson and Donaghy 2001).

2.6.2 Plant persistence and survival

It is understood that pastures are dynamic ecosystems, especially when coexist more than one dominating species within the pasture. Both, tiller appearance rate and tiller mortality rate, are a function of the climatic conditions, season, management (Bullock et al., 1994; Hernández-Garay et al., 1997b) and the species genetics (Volaire et al. 1995; Turner et al., 2006a,b,c,d). The understanding of tiller dynamic under field conditions would lead to a better management strategy to enhance the persistence and survival of the grass species (Matthew et al., 2000). At the grass tiller level, the increase in frequency and intensity of defoliation decreases tiller development (Donaghy and Fulkerson et al.,1998) and this varies with the species, phenological development and its association with environmental conditions (Langer, 1972; Bullock et al., 1994; Hernández-Garay et al., 1997). Also, a low grazing frequency reduces tiller density, however, it increases tiller mortality due to the shading generated by the taller tillers (Grant et al., 1983), but with an increase in content and/or concentration of WSC in the stubble (Donaghy and Fulkerson et al.,1997, 1998).

Once soil variables do not constrain plant growth, it is hypothesised that light becomes the environmental variable that limits pasture growth, thus light competition increases (Tilman and Downing, 1994). In a grazing system this is partially true, due to plant defoliation, which reduces light competition, nevertheless once the plants start to increase in leaf area the competition for light resource increases. A more frequent defoliation causes a photo-morphologic response, triggering shorter leaves and a greater tiller density (Matthew, 1996; Lemaire, 2001). In other words, there exists a size/density compensation mediated by grazing and defoliation criterion (Matthew, 1996). Therefore, defoliation frequency can regulate light competition and light utilisation by species, and it is especially important to maximise pasture growth by improving pasture light utilisation (Matthew, 1996), but it is necessary to reach a balance between the light utilisation with the increase of the plant energy reserves (WSC) to guarantee the plant regrowth. Therefore, there is a point in which increasing defoliation frequency and intensity cannot be compensated by increasing tiller population, thus the pasture begins to degrade by increasing plant death and diminishing tiller density, due to the depletion of the plant energy reserves (Donaghy and Fulkerson, 1997, 1998).

2.7 The effect of water restriction on plants

Drought has been described as the main abiotic stress that affects plants and crop production (Hu and Xiong, 2014) and is defined as the appreciable precipitation diminishment over an extended period, followed by a decreasing of soil moisture injuring the plants in the ecosystem (Kramer and Boyer, 1995).

Plants have developed adaptations over time to survive drought conditions, which can be inserted in two concepts, drought avoidance and drought tolerance. Drought avoidance includes all the adaptations in relation to morphological changes, such as, changes in leaf

area, orientation, and also the increasing resistance to water loss through cuticular resistance (Morgan, 1984, Zlatev, 2005), stomata control (Quarrie and Jones, 1979; Schapendonk et al., 1989; Galmes et al., 2007) and dormancy mechanism (Norton et al., 2008, 2006). Drought tolerance has been subdivided into two mechanisms, osmotic adjustments and changes in cellular elasticity, both mechanisms have the objective to maintain the cell turgor, keeping the cell metabolism functioning during drought conditions (Munns 1988; Blum, 2005; 2009; 2017).

Species with a greater root proportion reaching deep soil layers survive better (Blum, 2009; Neal et al., 2012; Ordóñez et al., 2018) and reach greater biomass production during water restrictions due to a higher water availability (Blum, 2009; Ordóñez et al., 2018) to fulfil its physiological and growth requirements (Blum, 2009).

Osmotic adjustments increase the survival during water restriction keeping the metabolic functions working during that period (Munns, 1988; Blum, 2005; 2009; 2017; Chimenti et al., 2006). The increase of osmotic potential (solute accumulation inside the cell) enhances the capacity of the plant to uptake soil water retained to a certain matric potential (Chimenti et al., 2006) increasing the available water for the plant. For example, Descalzi et al. (2019) showed *L. perenne*, *B. valdivianus*, *D. glomerata* and *H. lanatus* L. were able to survive, persist and grow in severe soil water restriction conditions, over the permanent wilting point (PWP; 1.5 MPa) between 0-20 cm of soil depth. This may be caused by higher levels of osmotic potentials inside the plant cells, as was indicated by Chimenti et al. (2006). The two strategies described allow the plant to survive, persist and increase the productivity across the water restriction. Plants have other adaptations as well, such as, accumulation of a higher concentration or content of WSC, prolonging the plant survival during a long period of water restriction (Volaire et al., 1995).

2.8 Water use efficiency during soil water restriction

Water use efficiency (WUE) is primarily defined as an amount of biomass produced by a certain quantity of water (Figure 2.3; Briggs and Shantz, 1913). This concept has become more important on agriculture due to the effect of global warming (IPCC, 2014).

Different levels can be used to evaluate WUE, the leaf, plant or crop levels. The evaluation at leaf level, are focus in photosynthesis rate and transpiration (Blum, 2005; 2009; Poni et al., 2009; Medrano et al., 2015); at plant level, the plant biomass with water loss (Medrano et al., 2015); and at crop level, the crop yield together with water utilised by the crop are the factors considered (Poni et al., 2009; Medrano et al., 2015). Differences between the scale of measurement have been detected (Poni et al., 2009; Medrano et al., 2015), but a higher sensitivity to detect stress is necessary (e.g. net photosynthesis and stomatal conductance), that way allow the quantification of the water stress effect on the plant, and can be detected during the moment that it is occurring as opposed to other methods, which are based on yield and biomass production.

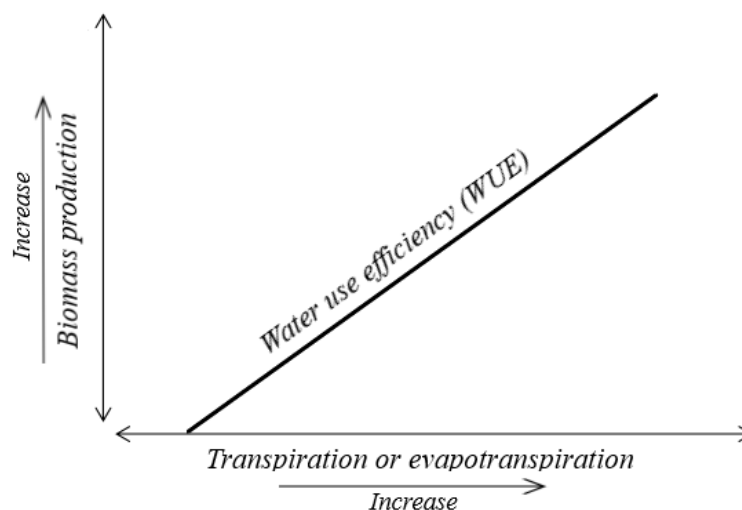


Figure 2.3 Adapted from Hatfield and Dold (2019). Water use efficiency concept, as the slope between biomass production and evapotranspiration.

Basso and Ritchie (2018) and Hatfield and Dold (2019) reported that greater WUE is related to a greater biomass production Figure 2.3, therefore, species with traits focus on increasing this parameter would increase the biomass production under different soil moisture conditions, this affirmation could be true under non-water deficit conditions. During water restriction periods an increase of WUE is related to a diminish in stomatal conductance and therefore, net photosynthesis. Blum (2009) argued that a higher WUE is not related to an increase in production during water restriction, because a higher WUE is related to a low stomatal conductance, and therefore, a low soil water uptake by the plant, provoking an increase of evaporation from the soil, which is water that will not be used by the plant for growth. This whole process finishes with a low biomass production during soil water restriction. On the other hand, deep-rooted species have a higher water availability (Neal et al., 2012; Ordóñez et al., 2018), and therefore, the stomata will remain opened for a longer period, producing more photo-assimilates and faster growth during the soil water restriction periods.

2.9 The effect of waterlogging on plants

Under flooded soils conditions with a slow drainage (limited gas diffusion), the main stress for plants is the lack of oxygen available in the soil for the roots (Drew, 1997). This is induced once the soil air pores (between 0 and 6 kPa of matric potential) are filled with water. The lack of oxygen, or hypoxia, has a direct effect on the plant metabolism, damaging the cell because of cytoplasmatic acidosis and the increase in toxic metabolites (Drew, 1997).

Plants generally have two strategies when are submitted to waterlogging, which generate stress by hypoxia. The strategies to survive this stress are divided in hypoxia tolerance and hypoxia avoidance (Drew, 1997; Sigua et al., 2012; Ploschuk et al., 2017). Hypoxia

tolerance is focus on metabolic adaptations and are different among the species and can be observed when the plant decreases its stomatal conductance to stop the leaf water loss, causing a decrease in the root hydraulic conductance, slowing down photosynthesis rate and respiration (Ploschuk et al., 2017). Hypoxia avoidance focuses on oxygen delivery to the root system (Sigua et al., 2012) and can be observed when the plant incorporates a structure called aerenchyma, which improves the aeration between and within the roots and/or shoots (Colmer, 2014; Takahashi et al., 2014). This structure helps the gas diffusion between endodermis and the surrounding environment (Drew, 1997).

Ploschuk et al. (2017) reported that *D. glomerata* and *Bromus catharticus* Vahl. mainly had root growth arrest, leaf senescence and a progressive stomata closure, decreasing the stomatal conductance, and therefore, photosynthesis when affected by waterlogging. The root mass diminishment due to waterlogging (hypoxia) was also observed by Etherington (1984), Stewart (1996) and McFarlane et al. (2003), who indicated that the same species of *Bromus* have low tolerance to hypoxic conditions.

2.10 Species general features

2.10.1 *Bromus valdivianus*

Bromus valdivianus is a native to the Temperate Humid climate regions of the south of Chile (Muñoz, 1980; Stewart, 1996; López et al., 1997), being one of the main species in the permanent pastures of those regions, which increases the pasture biomass production during the summer water restriction period (Lopez et al., 2013; Keim et al., 2015). Compared to *L. perenne*, it has a larger root system with greater rooting depth, i.e. 60 cm soil depth (Ordóñez et al., 2018). Annual accumulated herbage mass production of 13.1 t DM ha⁻¹ year⁻¹ has been reported (Balocchi and López, 2001), with forage quality comparable to *L. perenne* (López et al., 2013). *Bromus valdivianus* dominates deep free

draining soils, with medium texture and high levels of soil fertility (López et al., 1997). It can develop high growth rates during spring and summer, due to its drought tolerance, with a moderate growth during winter (Stewart, 1996; Balocchi and López, 2001; Keim et al., 2015), but is susceptible to waterlogging (Stewart, 1996). It naturally cohabits pastures with fast growing species such as *L. perenne*, and exhibits good competitive ability (Descalzi, 2011; Ordóñez, 2013) with similar nutritional requirements to *L. perenne*, both species competing under similar site and ecosystems conditions (López et al., 1997).

2.10.2 *Lolium perenne*

Lolium perenne is a species that grows in cool temperate regions around the world (Lamp et al., 1990). Under field conditions, a higher root proportion concentrates in the first 10 cm soil depth (shallow-rooted species), therefore, it has low growth rates during water restriction periods (Crush et al., 2005; Ordóñez et al., 2018), contributing to its energy adjustment during water stress periods, a strategy that improves plant survival (Nie and Norton, 2009).

The herbage mass production is around 9 - 15 t DM ha⁻¹ year⁻¹ in Southern Chile (Ruiz, 1988) and about 13 - 18 t DM ha⁻¹ year⁻¹ in New Zealand (National Forage Variety Trials, 2010; Crush et al., 2006) depending on the annual rainfall distribution and temperature. *Lolium perenne* grows one leaf at a time, having a maximum number of three live leaves at the same time. The defoliation criterion determined for *L. perenne* that enhances production and plant survival is to defoliate between 2 to 3 leaf stage, i.e. 2 or 3 fully expanded leaves (Donaghy and Fulkerson, 1997, 1998). The upper limit (defoliation at 3 leaf stage) was defined as the moment that the fourth leaf appears, and the first leaf starts to senesce.

2.10.3 *Plantago lanceolata*

Plantago lanceolata is a perennial dicotyledonous rosette plant (Sagar and Harper 1964) with large leaves (Stewart, 1996). Tolerant to drought due to its deep root system, combined with its high temperature tolerance (Sagar and Harper 1964; Stewart, 1996; Cranston et al., 2016) probably explained by its greater optimal growing temperature (Niu et al., 2006). This was observed by Pellissier (2014), reporting that *P. lanceolata* grew better under average temperatures of 20 °C than 12 °C. Its growth suppression by the average temperatures of 12 °C, was probably due to the significant diminishment of its photosynthesis rate, further down its metabolic optima temperature (Niu et al., 2006). Plants suffer photosynthetic inhibition when the temperature reaches extreme values upward or downward of their optima temperature (Xu et al. 2011). *Plantago lanceolata* has shown a greater herbage mass production than *L. perenne* and similar nutritive value (Rollo et al. 1998; Glassey et al. 2013; Cranston et al., 2015). The cultivar Tonic (used in this thesis) has the capacity to remain erect.

2.11 Summary

In the last three decades the ecosystem and pasture stability have gained attention due to the current global warming scenario and the negative effects of the intensive agriculture over the natural resources (i.e. loss of biodiversity, soil erosion, underwater contamination, amongst others). For this reason, the interest in ecological concepts such as resilience, resistance and stability started to be applied into the pastoral and agriculture productive systems.

The pastoral productive systems differ from the others agricultural activities due to the animal effect over the pasture and all the decisions involved around the grazing and pasture management. Besides, the research indicates that the regrowth of the species

under a vegetative or reproductive stage of development, the number of species and functional groups present in a pasture also are positively or negatively affecting the persistence, survival and biomass production of the pasture itself.

The plant energy reserves used for regrowth after defoliation is a relevant parameter to ensure the persistence and survival of the species over time. Thus, using a grazing criterion that allows the plants to replenish its energy reserves before the next grazing ensures a greater plant population, fast regrowth, greater root growth and persistence of the perennial species.

The functional groups in a pastoral ecosystem can be assessed by defining the functional traits of the individual species within the pasture. This parameter is a relevant driver of the ecosystem productivity, resilience, and resistance against environmental constraints. A higher pasture functional diversity within leads to a niche partitioning of the resources and to an asynchrony of growth. Nevertheless, there are few grazing studies on this topic performed under productive conditions.

Water availability for pasture growth and persistence is heading to be a major problem. The increasing severity of drought events together with the temperature, making more unpredictable the response of the rainfed pasture ecosystems against these conditions. Therefore, the understanding of the plant's capacity to uptake the water from the soil, its physiological and morphological responds during water restriction periods are important variables to consider in any research related to rainfed pastoral productive systems.

The literature review gives us several insights about the gaps related to pasture ecosystems, such as the lack of knowledge about *B. valdivianus*, which according to previous research, it show to be a good prospect for pasture production specially for increase summer pasture production; the lack of knowledge about the potentiality of the

species for water uptake under real productive conditions; The misleading concept of water use efficiency and how is important to focus on traits that maximize the water uptake even if that mean a decrease of water use efficiency; The diversity experiments are usually perform without the animal grazing which could lead to a confusing conclusions.

All these insights are considered in the present thesis focus on the species *B. valdivianus*, *L. perenne* and *P. lanceolata*. All of them are high productive species and with different traits and tolerance mechanisms and abilities to deal with water stress.

Chapter 3: Experimental chapter

**Physiology of growth and defoliation response of *Bromus
valdivianus* Phil. (pasture brome)**

Summary

- Increasing summer soil water restriction events are related to climate change. The growth physiology of *B. valdivianus*, a fast growing, high-quality, water restriction tolerant grass, was determined. It was also investigated how defoliation, based on leaf stage, affected tiller growth and water-soluble carbohydrates (WSC) utilisation and accumulation.
- Defoliation frequencies applied were at leaf stage 2 (LS 2), leaf stage 3 (LS 3), leaf stage 4 (LS 4) and leaf stage 5 (LS 5), distributed in a randomised complete block design. Measurements were performed on *B. valdivianus* plants and marked tillers. Tiller number per plant, leaf area, leaf and sheath weights and WSC were measured.
- *Bromus valdivianus* maintained 6 live leaves with 3 leaves growing simultaneously, reflected from LS 4 onwards. The highest shoot growth rate and WSC accumulation were reached at LS-4 and LS-5. The highest tiller photosynthetic rate was reached between LS-3 and LS-4 while the highest stomatal conductance from LS-3 onwards. Plant foliage and tiller growth were maximised at LS-4, coincidental with the highest root growth rate.
- In conclusion, *B. valdivianus* is a 'six-leaf' species, with three growing leaves per tiller, and leaf senescence beginning at LS-4.25. Performing defoliation at LS-4 did not disrupt aerial development, however, defoliation at LS-2 and LS-3 diminished leaf expansion rate.

3.1 Introduction

In temperate climates, a decline in rainfall and a concomitant increase in temperature during summer cause an increase in the soil matric potential (Descalzi et al., 2019), which can be exacerbated by climate change (Harrison et al. 2016). Consequences of that are the diminishment of pasture growth rate (Matthew et al., 2000), species survival (Volaire and Thomas, 1995; Hernández-Garay, 1997a,b; Volaire, 1998) and pasture persistence (Volaire and Thomas, 1995; Hernández-Garay et al., 1997b).

Therefore, during the summer period, the soil matric potential in the first 10 cm closer to the surface can reach values greater than 15430 hPa, which corresponds to the permanent wilting point (Hartge and Horn, 2009; Ordóñez et al., 2018). Under these conditions, shallow-rooted grasses survival and growth can be compromised, i.e. *L. perenne* (Neal et al., 2012; Ordóñez et al., 2018). Plants with longer roots can access water in deep soil layers, increasing its tolerance and resilience to soil water restriction (Barkaoui et al., 2016).

Drought-tolerant species (e.g. high deep root distribution species such as *B. valdivianus*) can be incorporated into pastures to both, mitigate the decline in pasture growth under dry soil conditions and increase the pasture species diversity (Tilman and Downing, 1994; Tilman et al., 2006). *Bromus valdivianus*, a perennial grass species, with similar accumulated herbage mass and nutritive value to *L. perenne*, dominates pastures in non-irrigated fertile Andisol soils of southern Chile (López et al., 1997), often coexisting with *L. perenne*. It has been reported that *B. valdivianus* compared to *L. perenne* has tillers that are twice as heavy, with almost four times greater leaf area per tiller, a longer total lamina length per tiller, but only half the tiller number per plant (López et al., 2013). The high percentage of both species within the same pasture, measured by botanical composition

(Ordóñez et al., 2018; Descalzi et al., 2019), suggests that there is a functional compatibility between *L. perenne* and *B. valdivianus*. Probably, the different growth strategies that both species shown (Donaghy and Fulkerson, 1997;1998; Ordoñez et al., 2017) favoured the persistence of *B. valdivianus* during a soil water restriction, as it can obtain water from deep soil layers to continue growing (Keim *et al.*, 2015; Ordóñez et al., 2018).

After increasing the diversity in a pasture by including drought-tolerant species, which are the defoliation regimes and criteria to sustainable manage a diverse pasture? It is possible that a diverse pasture that comprises a number of species may differ in its defoliation requirements in relation to *L. perenne* pasture (e.g. Rawnsley et al., 2002; Turner et al., 2006 a,b,c,d; Turner et al., 2007a,b). To have a defoliation regime that favours the fast growing species within a pasture (Flores et al., 2017), it is relevant to determine defoliation targets based on physiological and morphological principles. Until now the defoliation criterion for a diverse pasture containing *L. perenne* has been based on *L. perenne* research, targeting defoliation between the 2- and 3-leaf *L. perenne* regrowth stage, with the 2-leaf stage representing the point at which plant energy reserves are replenished (down limit), and the 3-leaf stage (upper limit) representing the point at which leaf senescence begins (Donaghy and Fulkerson, 1997, 1998; Fulkerson and Donaghy, 2001). However, for a diverse pasture, the optimal defoliation for individual species may differ from that of *L. perenne*, which at times might negatively impact its production or persistence.

The aim was to determine *B. valdivianus* growth dynamics and the effects that defoliation at different stage of development have on *B. valdivianus* growth. To accomplish this objective, the concentration and content of water soluble carbohydrates (WSC) in the

tiller base (bottom 5 cm), along with leaf elongation, leaf number, tiller population, herbage mass, root mass, plant leaf mass and tiller weight were determined under different leaf regrowth stage (LS) development (expansion of sequential new leaves per tiller). Also, the photosynthesis rate and stomatal conductance of *B. valdivianus* was assessed.

3.2 Materials and methods

3.2.1 Experimental conditions and treatments description

The experiment was carried out in a glasshouse at Massey University's Plant Growth Unit, Palmerston North, New Zealand. The experiment was set up at the end of June 2016 and treatments were implemented between 28 August 2016 and 25 January 2017.

The temperature in the glasshouse was evaluated with 1-hour frequency at the same height level as the pots. The extreme temperatures were prevented by using the two temperature control systems of the glasshouse, temperatures higher than 25 °C automatically activated the glasshouse windows opening and also the ventilation system functioning.

A total of 68 pots of 10 L volume capacity were utilised. The pot substrate used was a mix of 50% soil (Manawatu silt loam) and 50% sand. All the pots were automatically irrigated 3 times per day with a tube system, meeting the water requirements of all plants, keeping the pots at field capacity (25% of the volumetric soil water content).

The plants were fertilised to maintain an adequate nutrient status; a short-term fertiliser was added at a rate of 100 g pot⁻¹ with a concentration of 15% nitrogen (N), 6.0% phosphorus (P), 11.6% potassium (K), 1.5% magnesium (Mg), 6.0% sulphur (S), 2.0% iron (Fe) and 0.5% manganese (Mn), along with a long-term fertiliser at a rate of 100 g pot⁻¹ and containing 15% N, 2.2% P, 8.3% K, 0.2% Mg, 1.5% S, 1.5% Fe, 0.3% Mn and 0.2% zinc (Zn).

The study had 4 treatments and 17 blocks, however, only 6 blocks were evaluated for all the continuous variables measurements, i.e. lamina extension. The other 11 blocks were managed and grown under the same conditions as those 6 blocks and used only as extra blocks at the end of the study when a large amount of herbage material was required, i.e. WSC content evaluation. The pots within each block had similar light and humidity conditions. In order to avoid incident light and temperature differences between treatments, within blocks the pots were randomly moved every two weeks.

In each individual pot, two *B. valdivianus* seeds were located at equidistance positions, six closed to the edge and two in the centre. After germination, only one plantlet at each position was left to grow. The evaluated variables at plant and tiller level were performed on plants located in the centre of the pots: lamina length, number of leaves, leaf area per tiller, leaf mass per tiller, tiller number per plant and herbage mass per plant.

To stimulate tillering, the plants were defoliated two times before the experimental period began (Turner et al., 2012). Each defoliation was performed when the plants reached an average of 15 cm height from the ground level. After the third defoliation, the 28 August 2016, the following treatments were applied: 1) defoliation at leaf stage 2 (LS-2), 2) defoliation at leaf stage 3 (LS-3), 3) defoliation at leaf stage 4 (LS-4) and 4) defoliation at leaf stage 5 (LS-5).

In *L. perenne* for example the “one leaf stage” (1 LS) was identified as a total fully expanded leaf (100%), the “two leaf stage” (2 LS) as a total of two fully expanded leaf (100%) and so on (Fulkerson and Donaghy, 2001; Turner et al., 2006a). But unlike *L. perenne*, *B. valdivianus* expands three leaves simultaneously (Ordóñez *et al.*, 2017). The leaves in expansion are a fraction of the fully expanded leaf size (100% of expansion or 1 LS). The scores used for those leaves in expansion were: 0.25 LS (25% of expansion),

0.5 LS (50% of expansion) and 0.75 LS (75% of expansion). Two of these three leaves began its growth during the prior defoliation; thus, one of them due to its limited growth was not considered in the analysis, and the other one growth until a 75% or 0,75 LS, thus, was not considered as a fully expanded leaf.

As the present research was centred on *B. valdivianus* growth and development, the effect of defoliation frequency on *L. perenne* growth was not considered due to the existence of previous publications that had clarified the effect of the defoliation frequency over its performance and water soluble carbohydrates content (Fulkerson and Slack, 1994a,b; Donaghy and Fulkerson, 1997; 1998; Fulkerson and Donaghy, 2001; Turner et al., 2001; 2006a,b). However, extra pots of *L. perenne* were grown under the same conditions as those of *B. valdivianus* and used as known parameter for the LS development. The defoliation of *L. perenne* was performed at the same time as the *B. valdivianus* LS-5 treatment.

When the LS-5 treatment occurred for the third time, the other treatments were sequentially defoliated according to their corresponding leaf stage and the experimental period ended. For this reason, every treatment had different total accumulated growing days. The final harvest began with LS-5 on 30 December 2016 (124 days of growth from start of experiment), followed by LS-2 on 5 January 2017 (130 days of growth), LS-3 on 7 January 2017 (132 days of growth) and LS-4 on 26 January 2017 (151 days of growth). The leaf expansion and all the frequency defoliation cycles were calculated based on the accumulated growing degree days (C° day) with a base temperature of 5°C (McMaster and Wilhelm, 1997).

3.2.2 Evaluated variables

At the beginning of the experimental period, three tillers in the centre of each plant (two plants giving a total of six marked tillers per pot) were marked by placing a small coloured wire at their base (Poff et al., 2011) and every two days the leaf appearance, leaf number, lamina elongation and senescence were recorded. A new leaf was recorded as appearing when the tip of the new lamina was visible within the previous leaf sheath (Wilhelm and McMaster, 1995), and a leaf was considered fully expanded when its growth stopped, reaching its maximum length. The lamina length was measured as the distance between the lamina tip and collar section. For lamina length, only the green part of a lamina was considered, thus when a lamina began senescence, by decolouring from the tip to the ligule, the decoloured part of the lamina was not considered for lamina length measurements, in this way it was possible to determine lamina senescence rate (Duru and Ducrocq, 2000).

When a treatment reached the desired LS, the marked tillers were evaluated. First they were cut to 5 cm height above ground level and individually evaluated for leaf area using a LI-3100C area meter (LI-COR, Nebraska, USA; Accuracy between 1 – 5% using a resolution of 0,1 mm²) and dry leaf mass, after drying in an oven at 70°C for 48 hr. Following this, the remainder of the plant containing marked tillers (the two plants in the centre) was cut to 5 cm height and herbage collected, dried in an oven at 70°C for 48 hours to obtain mass on a dry matter (DM) basis. The plants located close to the edge of the pots were also cut to 5 cm height, and the herbage discarded.

At the final harvest, the number of tillers/plant were counted and recorded, and then the marked tillers were cut first to 5 cm height as previously described, and then to ground level (tiller base) and dried at 70°C for 48 hr. The 11 extra blocks ensured that the

minimum amount of dry matter herbage from tiller bases required was reached to measure WSC. Therefore, at the final harvest of the study, the tiller base of all non-marked tillers along with the tiller bases from the additional plants maintained in the 44 extra pots were harvested to ground level. WSC was analysed using Gas-Liquid chromatography (Holligan and Drew, 1970). To assess the root mass the soil was washed from the roots. Afterwards the roots of the two centre plants were dried at 70°C for 48 hr and weighed.

Net photosynthesis (A_n) and stomatal conductance (G_s) were evaluated in the LS-5 treatment using four fully expanded leaves and one growing leaf prior to the defoliation of LS-5 treatment in order to assess the effect of leaf age. For this evaluation leaf 1 was the oldest leaf and leaf 5 the youngest leaf. The evaluation used a LICOR-6400XT (LI-COR, Nebraska, USA), with a 6400-02B LED light source chamber (LI-COR, Nebraska, USA). The photosynthetic photon flux density at the leaf surface was set at 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, leaf temperature was stabilised at 25°C, a flow rate of 500 $\mu\text{mol s}^{-1}$ was set and the ambient CO_2 concentration of the incoming gas stream was set up at 400 $\mu\text{mol s}^{-1}$. Net photosynthesis (A_n) and stomatal conductance (G_s), were determined in two dates, 10 November 2016 and 22 December 2016, just before the harvests. For each of these variables, two leaves of the same age were set inside the chamber and leaf area was determined for each one using a digital calliper.

In order to assess the effect of defoliation frequency on plant regrowth rates of the following parameters were determined, leaf elongation, leaf area growth, shoot growth and root growth. For the latest three parameters named, accumulated growth was divided by the total growing days for each treatment as follow: LS-2 (130 days of growth), LS-3 (132 days of growth) and LS-4 (151 days of growth), LS-5 (124 days of growth). 1) *Leaf elongation*: The elongation rate was determined with the first expanded leaf of each

treatment (leaf 3). For leaf elongation quadratic equations were determined. The slope determined which defoliation treatments had a faster leaf elongation after the third day post-defoliation. The calculation of the slope for all the equations was done considering 50 accumulated growing degree days (third day), as this is the moment when WSC have a greater influence on leaf regrowth (Donaghy and Fulkerson 1997; Turner *et al.*, 2001).

2) *Leaf area growth rate*: The accumulated leaf area of all the defoliations was divided by the total of growing days for each treatment, allowing determination of the growing rate of the tiller leaf area ($\text{cm}^2 \text{ day}^{-1}$). 3) *Shoot growth rate*: The accumulated plant herbage mass harvested in each defoliation event (above 5 cm) was divided by the total growing days of each treatment, allowing determination of the shoot growth rate (g day^{-1}). 4) *Root growth rate*: The total root mass was divided by the total number of growing days of each treatment, allowing determination of the root growth rate (g day^{-1}).

In order to evaluate the phyllochron and the growing degree days required for a fully expanded leaf, the leaf length data collected every two days were used. An average between each leaf appearance was determined and the phyllochron calculated. As *B. valdivianus* leaf 4 is the first fully expanded leaf (Ordóñez *et al.*, 2017), this leaf was used to calculate the growing degree days necessary for a leaf to reach full expansion.

3.2.3 Experimental design and statistical analysis

The experiment was laid out as a randomised complete block design (4 treatments x 6 blocks). The normality of the data was evaluated using the Shapiro-Wilk test ($p \leq 0.05$). Tiller weight and tiller leaf area were normalised by using natural logarithm function, and together with normal distributed data, such as phyllochron, leaf area growth rate, tiller leaf weight, net photosynthesis, stomatal conductance and intercellular carbon concentration, were analysed using an analysis of variance (ANOVA). Treatments effects

were separated by applying Fisher's least significance difference test (LSD) ($p \leq 0.05$). It was not possible to normalise tiller number, plant shoot growth rate, root growth rate, and plant and tiller WSC (fructose, glucose and starch), using functions transformation, therefore non-parametric statistics were applied (box diagrams, mean, median, percentiles, and standard deviation) and the Kruskal-Wallis's test ($p \leq 0.05$) was used to compare the treatment means. Canonical variate analysis (CVA) was performed to explain the differences between the treatments and their variation, based on the joint analysis of all the variables and data. It was undertaken on the standardised data, thus each variable had average zero and standard deviation equal one (Marsh and Elliott, 2008).

3.3 Results

3.3.1 *Bromus valdivianus* growth dynamic

When the treatment LS-5 reached the third defoliation, all the following treatments were consecutively harvested as they were achieving its corresponding LS development. LS-2 completed 8 cycles; LS-3, 6 cycles; LS-4, 5 cycles; and LS-5, 3 cycles (Figure 3.2).

Bromus valdivianus maintained six live leaves simultaneously: three fully-grown leaves (Figure 3.1: leaf 2, 3 and 4; Figure 3.2), and three actively growing leaves (Figure 3.1: leaf 5, 6 and 7; Figure 3.2). The first leaf (Leaf 1), indicated in Figure 3.1, is considered a 'residual leaf', being the last expanding leaf at the time of the prior defoliation event and was not included in this analysis. Leaf 2 was a younger 'residual leaf' than Leaf 1 from the prior defoliation event, it grew until 75% of the total length of a complete normal lamina, being considered with a score of 0.75 LS. The other two leaves that reached full expansion were leaf 3 and leaf 4, scored as 1 LS each.

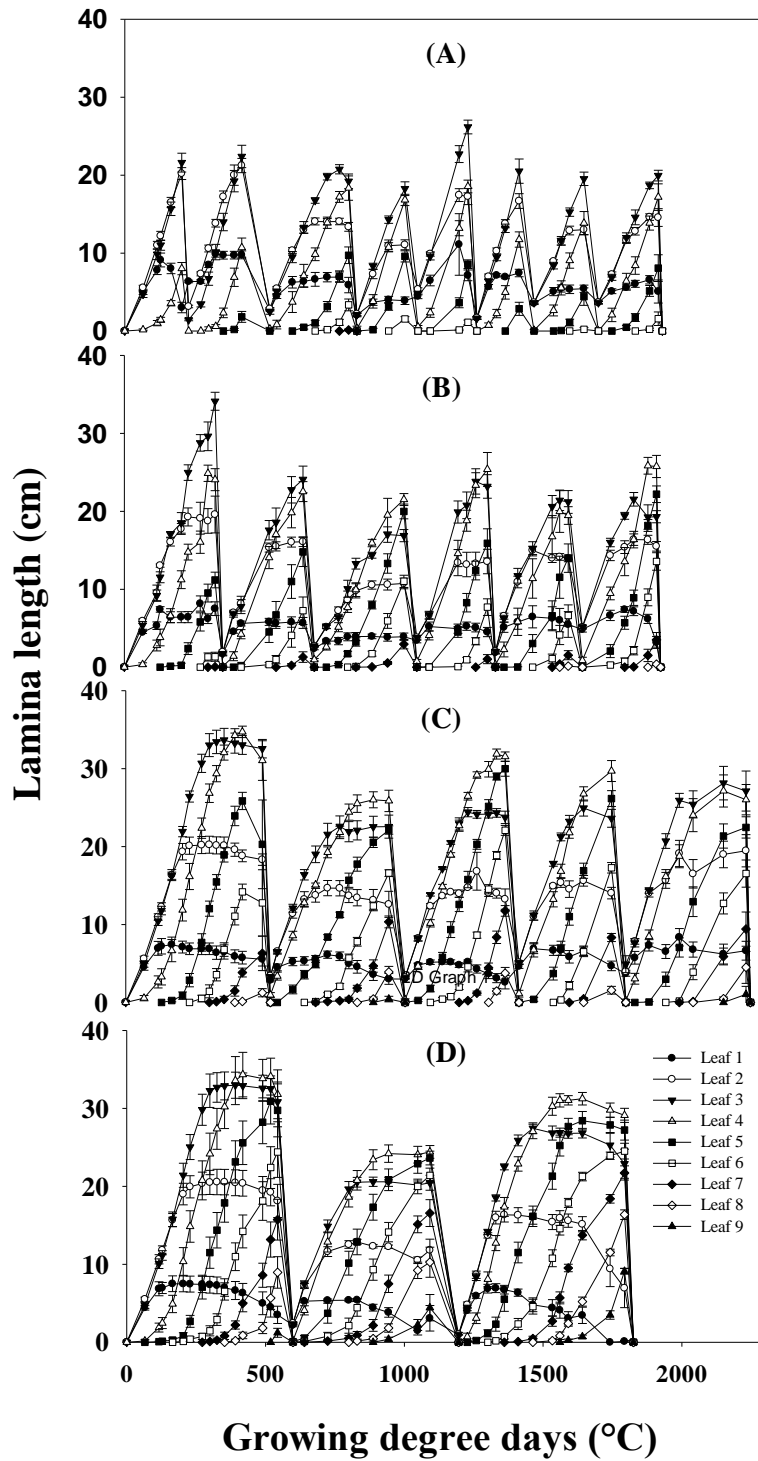


Figure 3.1 Leaf growth dynamic and regrowth periods for each defoliation treatment for *B. valdivianus*. Defoliation at LS-2 (A); Defoliation at LS-3 (B); Defoliation at LS-4 (C); Defoliation at LS-5 (D). Bars indicate the standard error of the mean (n = 6).

When leaf 8 appeared, the oldest leaf (Leaf 2) started to senesce. As senescence began, the tillers had three completely expanded leaves, second, third and fourth leaf (0.75 LS + 1LS + 1 LS) and three expanding leaves, fifth, sixth and seventh leaf (0.75 LS, 0.5 LS and 0.25 LS). Thus, *B. valdivianus* leaf senescence started at 4.25 LS (Figure 3.1). Phyllochron was not affected by the defoliation frequency ($p>0.05$) and on average it took 96°Cd for one leaf to appear. The leaves reached full expansion at 431°Cd accumulated growing degree days after leaf appearance, but with 3 leaves growing at the same time.

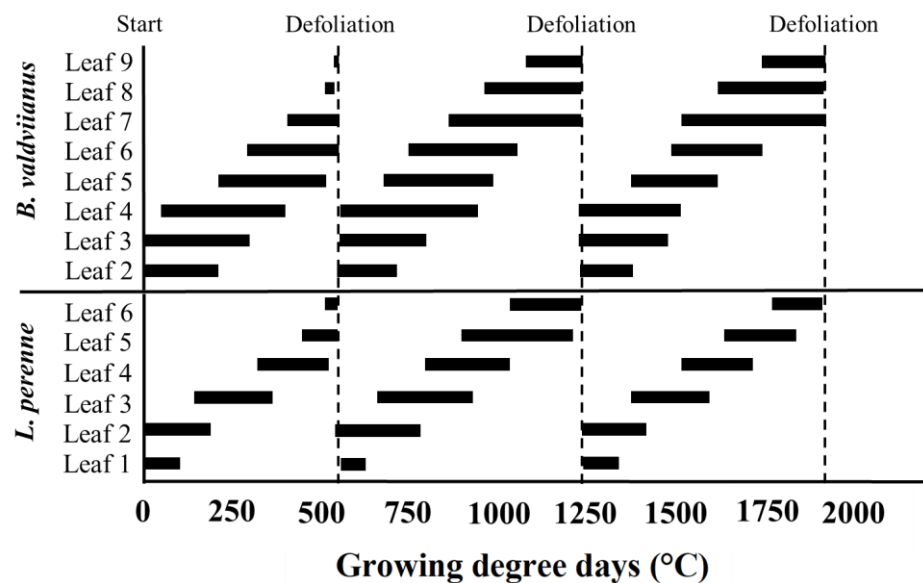


Figure 3.2 Scheme of the leaf expansion cycles based of AGDD for the LS-5 treatment of *B. valdivianus* and the extra treatment of *L. perenne* based on the *B. valdivianus* (LS-5) defoliation frequency.

Figure 3.2 shows the differences between *B. valdivianus* and *L. perenne* in leaf stage development, lamina length expansion and leaf appearance over time, expressed as accumulated growing degree days. Also, from Figure 3.2 can be extracted the accumulated growing degree days needed for a full expanded leaf.

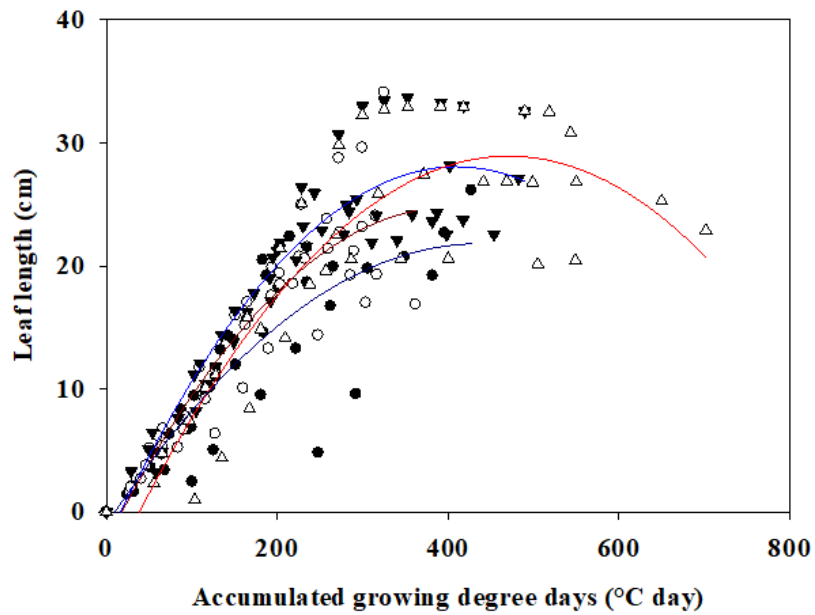


Figure 3.3 Growth rate of the first fully expanded lamina (Leaf 3) from each defoliation frequency: leaf stage 2 (Dark blue line; filled circle), leaf stage 3 (brown line; empty circle), leaf stage 4 (clear blue line; filled triangle) and leaf stage 5 (red line; empty triangle). The equations are for LS-2) $y = -0.0001x^2 + 0.1045x - 1.0023$ ($R^2 = 0.71$); LS-3) $y = -0.0002x^2 + 0.135x - 2.3488$ ($R^2 = 0.83$); LS-4) $y = -0.0002x^2 + 0.1489x - 2.4398$ ($R^2 = 0.87$); LS-5) $y = -0.0002x^2 + 0.1456x - 5.3445$ ($R^2 = 0.80$).

When defoliation was performed at LS-5, *B. valdivianus* presented 9 leaves; and when its 7th leaf appeared the 2nd leaf started to senesce (Figure 3.1). *Lolium perenne* under the same frequency of defoliation, that was dependent on *B. valdivianus* reaching LS-5, generated 6 leaves (Figure 3.2) and when its 4th leaf appeared the 1st leaf began to senesce.

The effect of defoliation frequency on plant and tiller growth was evaluated through the following variables:

1) *Leaf elongation*: Figure 3.3 shows the first fully expanded leaf (Leaf 3 in Figure 3.1). The growth rate of Leaf 3 was retarded at the beginning of its growth, by the more frequently defoliation criterion. The derivation of the quadratic equations (Figure 3.3) replacing the X as AGDD (accumulated growing degree days) with 50 AGDD (third day after a defoliation event), ordered the slopes as follow: LS-4 (0.1289) > LS-5 (0.1256) > LS-3 (0.115) > LS-2 (0.0945).

Table 3.1 Plant parameters at the final harvest and the accumulated over all the defoliations under different defoliation frequencies.

Final harvest results			Sum of all defoliations		
Defoliation treatments	Tiller leaf area (cm ² tiller ⁻¹)	Tiller weight (g tiller ⁻¹)	Tiller leaf weight (g tiller ⁻¹)	Tiller leaf area growth rate (cm ² tiller ⁻¹ day ⁻¹)	Phyllochron (°C Day)
LS-2	3.56 c (12.7 cm ²)	0.24 d (0.134 g)	0.11 d	38.76	101.4
LS-3	4.85 c (23,9 cm ²)	0.33 c (0.25 g)	0.22 c	39.93	92.9
LS-4	5.30 b (28.4 cm ²)	0.49 b (0.48 g)	0.30 b	44.98	94.4
LS-5	6.62 a (44.3 cm ²)	0.57 a (0.60 g)	0.35 a	35.97	95.5
p-value	≤0.001	≤0.001	≤0.001	>0.05	>0.05
SEM					
LS-2	0.058	0.004	0.006	2.5	2.4
LS-3	0.281	0.027	0.008	3.1	4.2
LS-4	0.260	0.018	0.009	3.5	2.0
LS-5	0.286	0.034	0.027	1.8	2.5

Note: Final harvest data was collected during that specific harvest: Sum of all defoliation is the accumulated outcome considering all defoliation of each treatment. The data in parenthesis is the value without normalization. SEM is the standard error of the mean (n = 6) with 6 subsamples per repetition. LS-2 (defoliation at leaf stage 2); LS-3 (defoliation at leaf stage 3); LS-4 (defoliation at leaf stage 4); LS-5 (defoliation at leaf stage 5).

2) *Leaf area growth rate*: no statistical differences between the treatments were found (p>0.05; Table 3.1).

3) *Plant shoot growth rate*: Statistical differences were determined ($p \leq 0.001$; Figure 3.4), where the results of shoot growth rate were as follows (mean \pm sem): LS-5 0.23 g day^{-1} ($\pm 0.017 \text{ g}$) = LS-4 0.23 g day^{-1} ($\pm 0.017 \text{ g}$) > LS 3 0.18 g day^{-1} ($\pm 0.013 \text{ g}$) = LS-2 0.13 g day^{-1} ($\pm 0.008 \text{ g}$).

4) *Root growth rate*: Statistical differences were determined ($p \leq 0.001$; Figure 3.4) and were as follow (mean \pm sem): LS-5 $0.049 \text{ g day}^{-1} \text{ plant}^{-1}$ ($\pm 0.007 \text{ g}$) > LS-4 $0.018 \text{ g day}^{-1} \text{ plant}^{-1}$ ($\pm 0.003 \text{ g}$) = LS-3 $0.011 \text{ g day}^{-1} \text{ plant}^{-1}$ ($\pm 0.002 \text{ g}$) = LS-2 $0.008 \text{ g day}^{-1} \text{ plant}^{-1}$ ($\pm 0.001 \text{ g}$).

3.3.2 Water soluble carbohydrates reserves

Defoliation frequency resulted in significant differences ($p \leq 0.001$) in the tiller and plant WSC content. Glucose and fructose decreased with a more frequent defoliation (Figure 3.5). The median of the glucose content was as follows: LS-5 $109.3 \text{ mg plant}^{-1}$ LS-4 $56.3 \text{ mg plant}^{-1}$, LS-3 $30.1 \text{ mg plant}^{-1}$ and LS-2 $17.24 \text{ mg plant}^{-1}$; while the median of the fructose contents were as follow: LS-5 $206.1 \text{ mg plant}^{-1}$, LS-4 $113.2 \text{ mg plant}^{-1}$, LS-3 $42.06 \text{ mg plant}^{-1}$ and LS-2 $26.69 \text{ mg plant}^{-1}$. The median of the starch content followed a different pattern than glucose and fructose: LS-4 $11.81 \text{ mg plant}^{-1}$, LS-5 $8.95 \text{ mg plant}^{-1}$, LS-3 $3.00 \text{ mg plant}^{-1}$ and LS-2 $2.57 \text{ mg plant}^{-1}$. The median of the total WSC content followed a similar pattern as the glucose and fructose: LS-5 $329 \text{ mg plant}^{-1}$, LS-4 $209.03 \text{ mg plant}^{-1}$, LS-3 $82.14 \text{ mg plant}^{-1}$ and LS-2 $50.02 \text{ mg plant}^{-1}$.

As general trend, the treatments LS-5 and LS-4 had the same content of WSC and starch per tiller and per plant. The total WSC showed that the treatments LS-5 and LS-4 were statistically similar. Such a result was also obtained between LS-4 and LS-3. However, LS-3 was lower than LS-5, and similar to LS-2.

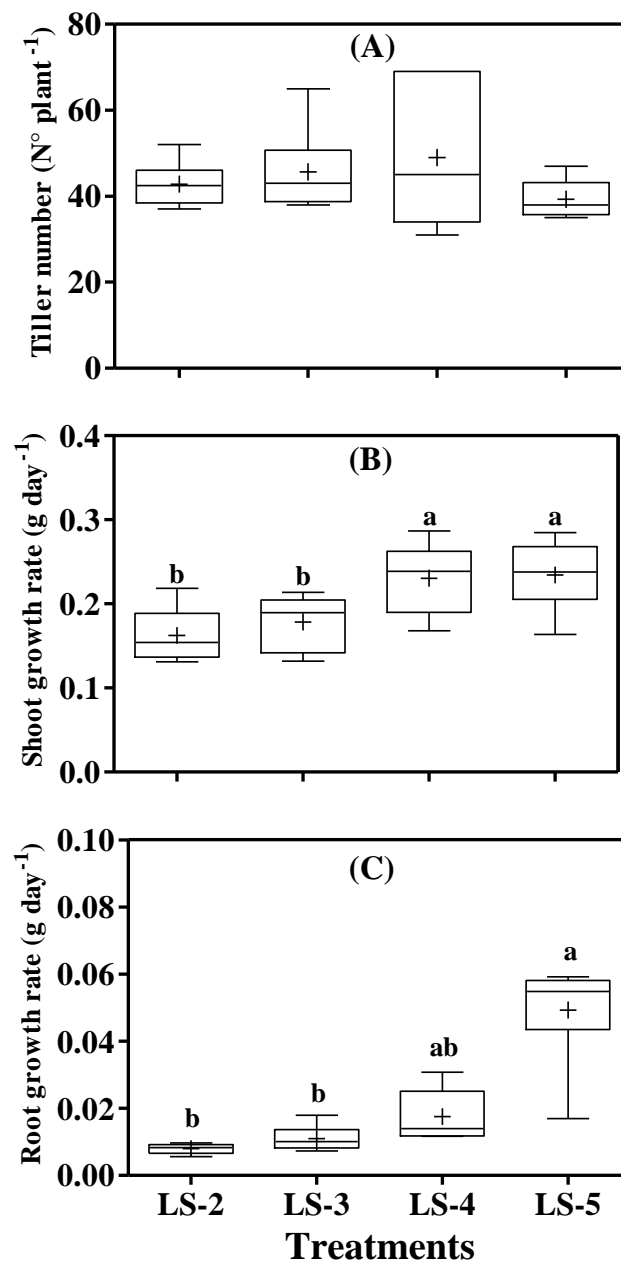


Figure 3.4 Tiller number (A), shoot growth rate (B) and root growth rate (C). Lowercase letters indicate difference between treatments. Lowercase letters indicate difference between treatments. + indicates the mean, bars and boxes the distribution of the population (quartiles) (n = 12).

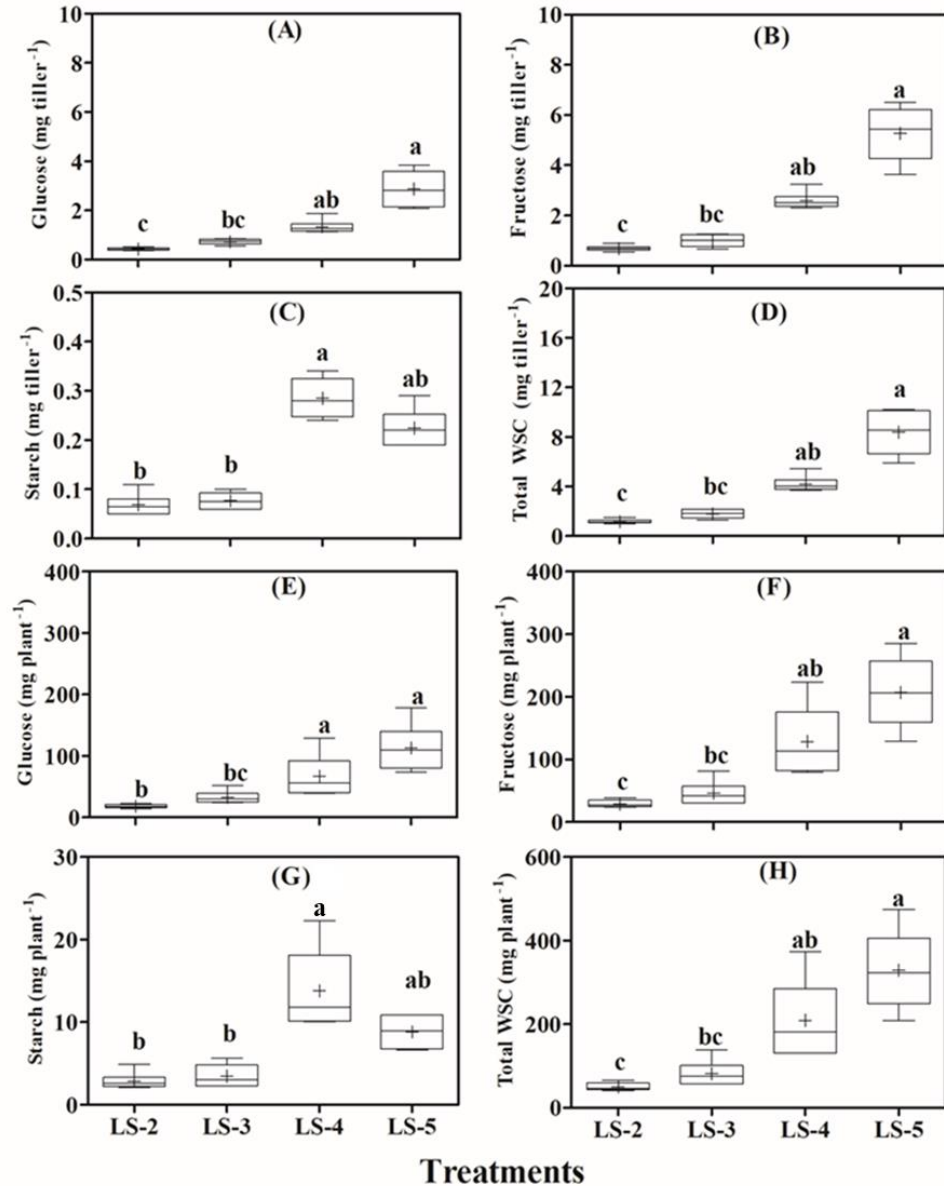


Figure 3.5 Water soluble carbohydrates (WSC), mg of glucose per tiller (A) mg of fructose per tiller (B), mg of starch per tiller (C), mg of total WSC per tiller (D), mg of glucose per plant (E), mg of fructose per plant (F), mg of starch per plant (G) and mg of total WSC per plant (H). The WSC content per plant was determined by multiplying the quantity of the WSC per tiller by the average of tiller population per plant determined at the end of the experiment. Lowercase letters indicate difference between treatments. + indicates the mean, bars and boxes the distribution of the population (quartiles) (n = 17).

3.3.3 Net photosynthesis and stomatal conductance

Table 3.2 Net photosynthesis (A_n) and stomatal conductance (G_s) for *B. valdivianus* during two evaluation dates at different leaf ages for the LS-5 treatment.

Leaf number	11 October 2017		22 December 2017	
	A_n ($\mu\text{mol cm}^2 \text{s}^{-1}$)	G_s ($\mu\text{mol cm}^2 \text{s}^{-1}$)	A_n ($\mu\text{mol cm}^2 \text{s}^{-1}$)	G_s ($\mu\text{mol cm}^2 \text{s}^{-1}$)
Leaf 1	7.56 c	0.039 c	7.04 c	0.088 c
Leaf 2	9.49 b	0.055 b	9.28 b	0.126 b
Leaf 3	10.93 a	0.083 a	11.41 a	0.192 a
Leaf 4	11.00 a	0.086 a	11.83 a	0.197 a
Leaf 5	8.97 b	0.098 a	10.37 b	0.160 a
P value	≤ 0.001	≤ 0.05	≤ 0.001	≤ 0.05
SEM				
Leaf 1	0.43	0.009	0.24	0.014
Leaf 2	0.66	0.007	0.39	0.012
Leaf 3	0.11	0.011	0.66	0.024
Leaf 4	0.87	0.018	0.53	0.014
Leaf 5	0.49	0.003	0.65	0.007

Note: A_n is net photosynthesis and G_s stomatal conductance of the last expanded leaf for the LS-5 treatment (defoliation at leaf stage 5). leaves are from 1 tiller per pot. SEM is the standard error of the mean ($n = 6$).

The analysis of net photosynthesis (A_n) showed that there were statistical differences in leaf ageing ($p \leq 0.001$), but not differences between dates, 11 October and 22 December of 2017 ($p > 0.05$). A_n decreased as leaf age increased, the exception being leaf 5 (the youngest leaf) with a lower net photosynthesis than leaf 4. Stomatal conductance (G_s) declined significantly with increasing leaf age ($p \leq 0.05$; Table 3.2). There was no significant interaction between date and G_s ($p > 0.05$).

3.3.4 Canonical variate analysis

The canonical variate analysis (CVA) shows the relationships between the measured variables and the defoliation regimes (Figure 3.6; Figure 3.7). The first two canonical

variates explained 98% of the differences between treatments (Wilk's Lambda: $p \leq 0.001$).

The first canonical variate (CAN 1; $p \leq 0.001$) explained 74% of the differences between the treatments and the second canonical variate (CAN 2; $p \leq 0.001$) explained 24%.

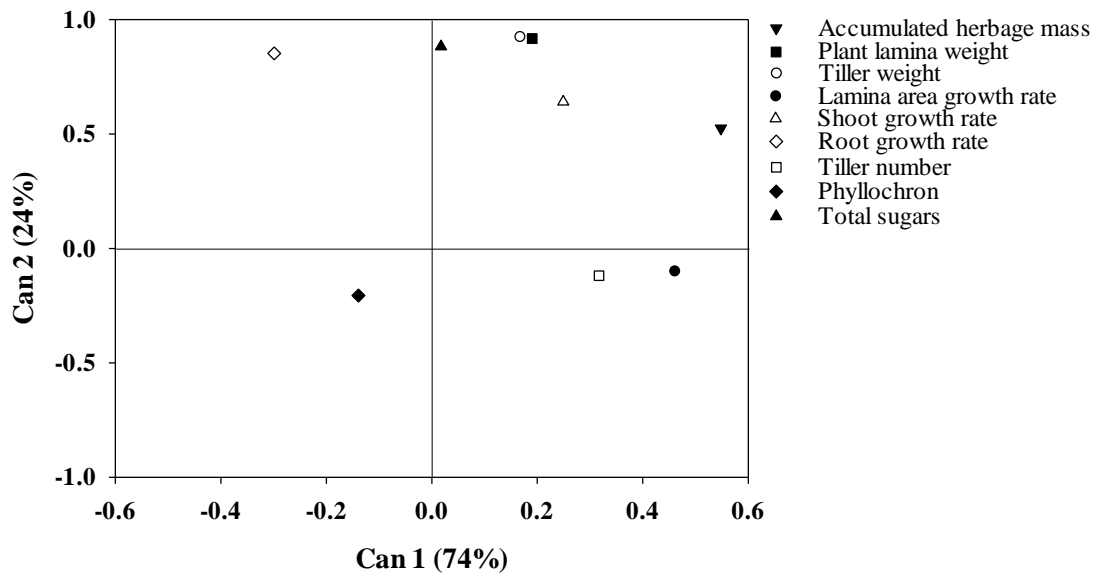


Figure 3.6 The effect of defoliation frequency over the plant leaf stage development evaluated through over plant growth and physiological parameters.

Accumulated herbage mass, lamina area growth rate, tiller number and shoot growth rate were positively associated to CAN 1, while root growth rate was negatively associated. Accumulated herbage mass, plant lamina weight, tiller weight, shoot growth rate, root growth rate and total sugars were positively associated to CAN 2, but no plant parameter was positively associated with CAN 2 in its negative direction.

The longer defoliation treatments LS-4 and LS-5 were more positively associated with the most of parameters related to plant growth than LS-2 and LS-3. Root growth rate and total sugars were more related to LS-5 than LS-4. All the positive plant parameters correlated with CAN 1 were more related to LS-4 than LS-5.

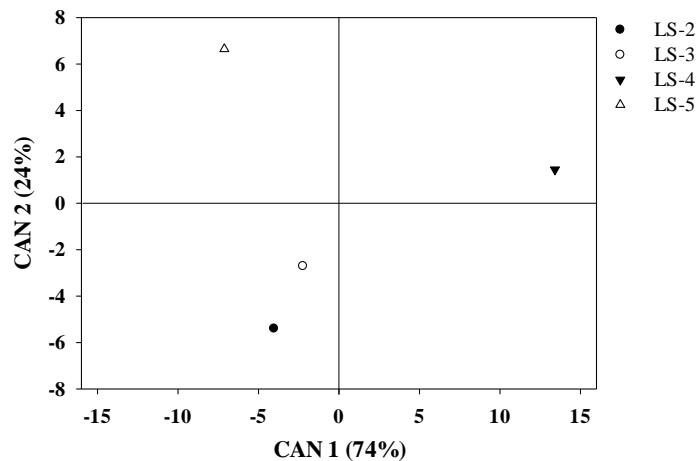


Figure 3.7 The differences between the treatments explained in the Figure 3.6. defoliation at Leaf stage 2 (LS-2), 3 (LS-3), 4 (LS-4) and 5 (LS-5).

3.4 Discussion

3.4.1 Leaf growth dynamic

The current study determined that *B. valdivianus* maintains six live leaves tiller⁻¹, with three of these totally expanded, and the remaining three simultaneously expanding. This is a major growth physiology difference between *B. valdivianus* and *L. perenne*, as *L. perenne* has only one expanding leaf at a time and maintains a total of three live leaves tiller⁻¹ (Fulkerson et al., 1994a; Fulkerson and Donaghy, 2001). *Dactylis glomerata* L. (cocksfoot) and *Bromus willdenowii* Kunth. (prairie grass) can maintain five live leaves tiller⁻¹ and grow two leaves simultaneously, when the first leaf reaches 75% of its total expansion length the next leaf appears (Turner *et al.*, 2007b). For *B. valdivianus*, when the first leaf reached its maximum expansion, the second leaf had reached 75% of its maximum expansion, the third leaf had reached 25% of its expansion, and the fourth leaf is starting to grow. Leaf senescence began with the leaf number 2 and started when tillers had accumulated 7 live leaves (i.e. LS-4.25).

A new *B. valdivianus* leaf appeared every 96°Cd (Table 3.1) and each individual leaf required 431°Cd for full expansion (Figure 3.1; Figure 3.2; Figure 3.3). These results contrasted with leaf parameters determined for other species, such as *L. perenne*, in which a new leaf appeared every 117°Cd (Davies and Thomas, 1983). It is important to consider that, even though *B. valdivianus* required a longer period to fully expand a leaf (431°Cd), three leaves expand at the same time. The strategy of *B. valdivianus* appears to be focused on leaf number, and tiller and leaf size more than tillering itself, a major difference of growth strategy if we compare *B. valdivianus* with *L. perenne*. It has been reported that *L. perenne* grown in a subtropical environment and defoliated with 3 live leaves at 5 cm height, had a tiller weight of 0.036 g, and plants averaged 86 tillers (Fulkerson and Slack, 1994b), leaf area around 2.7 cm² and had four times more tillers than *B. wildenowii*, when defoliated to 6 cm height (Hume, 1991). These results are opposite regarding to *B. valdivianus*, where, after several defoliations, the tiller population reached 41 tillers plant⁻¹, a tiller weight between 0.134 g - 0.6 g and leaf area between 12.7 cm² - 44.3 cm². The current study describes the growth strategy of *B. valdivianus* in terms of the number of live leaves maintained per tiller, the size and the lifespan of individual leaves, and the low tiller number per plant, which are all different strategies to those exhibited by *L. perenne*. These strategies have been successful in the south of Chile on Andisol soils, where according to Balocchi et al. (1996), both species coexist in the same grassland ecosystems, with both *B. valdivianus* and *L. perenne* strongly contributing to the pasture production.

3.4.2 Net photosynthesis and stomatal conductance related to leaf ageing

The total photosynthesis accomplished for the whole tiller for *B. valdivianus*, an average of A_n between the five measured leaves (Table 3.2) were determined, and it was 17.15

$\mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$ and according to the results the leaf area per tiller at LS-4 was 24.8 cm^2 , giving a total A_n per tiller of $0.0425 \mu\text{mol s}^{-1}$. This value is 8 times greater than *L. perenne*, assuming a leaf area of approximately $2.7 \text{ cm}^2 \text{ tiller}^{-1}$ at LS-3 (Hume, 1991), and an average A_n of $20 \mu\text{mol m}^2 \text{ s}^{-1}$ (Ainsworth et al., 2003), giving a total photosynthesis per tiller of $0.0054 \mu\text{mol s}^{-1}$. This greater *B. valdivianus* A_n per tiller over *L. perenne* could be one of the advantages of *B. valdivianus* in a mixed sward with *L. perenne*. For example, *B. valdivianus* could allocate more WSC to root growth, resulting in a higher survival rate during summer in comparison to *L. perenne* (López et al., 2013), due to a higher water extraction from deeper soil layers (Ordóñez et al., 2018).

The decrease in both A_n and G_s as leaf age increased (Table 3.2), has also been reported in potato leaves (Haimeirong and Kubota, 2003) and in maize leaves (Xu et al. 2008; 2011). This decrease in photosynthetic capacity has been related to several causes, such as the electron transport rate (Iacono and Sommer 2000; Xu et al., 2011), chlorophyll content (Ge et al., 2011) and carotenoids (Yoo et al., 2003). Negative effects that could be related to a lipid peroxidation induction by active oxygen species (AOS) (Cakmak and Horst, 1991; Gill and Tuteja, 2010). The increase of AOS is determined by the concentration of malondialdehyde (MDA; Xu et al., 2011) and the physical damage of cell membranes, evaluated through ion leakage (Xu et al., 2009; 2011). The cell damage generated by AOS increases with leaf age (Xu et al., 2009; 2011) and it could be one explanation for why photosynthetic capacity decreases with leaf age.

3.4.3 Effect of defoliation frequency on non-structural carbohydrates reserves

The current study supports that the defoliation frequency has a direct impact on the storage of WSC (Turner et al., 2006 a,b,c,d; Donaghy and Fulkerson, 1998) and on

subsequent plant regrowth (leaf growth rate) within the first three days after defoliation (Donaghy and Fulkerson 1997; Turner et al., 2001) on *B. valdivianus*.

Non-structural carbohydrates vary with day/night temperature, amount of daylight, levels of nutrients and water availability, which act to alter the relationship between respiration and photosynthesis (Fulkerson and Donaghy, 2001), for different grass species (Turner et al., 2006a,b,c,d; Turner et al., 2007a,b). Many researchers have investigated defoliation interval and its effect on WSC storage and subsequent regrowth and survival of various grass species (e.g. Alberda, 1966; White, 1973; Donaghy and Fulkerson 1997; Thornton and Millard 1997; Turner et al., 2001). Alberda (1966) postulated that 60 g WSC kg⁻¹ may be a critical value to maintain the cellular function of *L. perenne*. Donaghy and Fulkerson (1997) indicate that above 160 g WSC kg⁻¹, there is no longer an effect on regrowth of *L. perenne*. Turner et al. (2006a) recommend defoliation at LS-4 for *D. glomerata*, with a content of 218 mg WSC plant⁻¹, equivalent to 236 g WSC kg⁻¹. Turner et al. (2006d), also for *D. glomerata*, recommend defoliation frequency between LS-2 and LS-4, which corresponds to 70 mg WSC plant⁻¹ and 1,477 mg WSC plant⁻¹, respectively. Turner et al. (2007c) recommend defoliation at LS-4 for *B. wildenowii*, where the content of WSC was around 600 mg plant⁻¹. These results indicate how difficult an ‘optimum’ level of WSC is to define, especially when the grasses species have a different growth strategy. According to the current study, WSC reached levels of concentrations around 60 g kg⁻¹ through LS-2 to LS-4, increasing to 140 g WSC kg⁻¹ at LS-5. However, several authors have reported that WSC content (expressed as either mg tiller⁻¹ or mg plant⁻¹) is more significant than WSC concentration (expressed as % or g kg⁻¹), in regrowth and survival (e.g. Fulkerson and Slack, 1994b; Donaghy and Fulkerson, 1998; Turner *et al.*, 2006d). The latter is supported by the present study, where the WSC

content increases as a response of a more infrequent defoliation, together with the growth plant parameters.

Regarding starch, it is recognised that starch is the main storage product of many seeds, e.g. grasses (Tetlow *et al*, 2004). The seeds are developed in the stubble of the grasses, which explains the constant increase of starch until the treatment at LS-4, as the seeds were being developed from the first 5 cm of the plant. The LS-5 starch concentration diminishment was due its movement from the stubble to the inflorescence, which was occurring at that moment for that treatment (data not shown).

3.4.4 Effect of defoliation frequency on plant regrowth

In the current study, although there was no statistical difference in tiller number ($p>0.05$), at LS-5, *B. valdivianus* had a greater tiller leaf area, tiller weight and tiller leaf weight than all other treatments. When leaf expansion was analysed, the derivative of the growing curve at the third day of the growing period showed that LS-4 had a higher slope than LS-5. However, the growth rate at LS-5 was similar to LS-4 as shown in Figure 3.4B. Figure 3.4B also shows how defoliation at LS-4 resulted in the maximum growth rate, therefore, any earlier defoliation will negatively affect the biomass production (Hume, 1991) and probably plant persistence (Fulkerson and Donaghy, 2001).

In the current study, a decrease in WSC when defoliation frequency increased to LS-2 or LS-3 indicated a detrimental effect on plant recovery and plant shoot growth rate, root growth rate, tiller leaf weight and tiller leaf area. A similar effect of defoliation frequency on WSC levels has been reported for *L. perenne* (Fulkerson and Donaghy, 2001). It is important to highlight that LS-4 did not show a detrimental effect on WSC content (mg plant^{-1} or mg tiller^{-1}) and was statistically similar to LS-5. These results are also supported in the CVA, that show a high association between most of plant parameters evaluated are

highly related to LS-4 and LS-4 (CAN 1). The total sugars (in CAN 2) showed a high correlation with LS-5 followed by LS-4.

The impact of the frequent defoliations, besides the depletion of WSC in the stubble, also generate a slower regrowth in leaves, roots and tillers, affecting negatively its persistence within the pasture (Donaghy and Fulkerson, 1997,1998). The allocation patterns defined for *L. perenne*, after defoliation, were as follow, leaf regrowth, root regrowth and then tiller initiation. Therefore, tiller initiation and population (Donaghy and Fulkerson 1998) should be the first parameter to be affected. Contrarily, tiller number was not affected by the defoliation regimes (Figure 3.4A; Figure 3.6; Figure 3.7), whereas, root growth rate and shoot growth rate were affected by defoliation regimes. From this it could be hypothesized for *B. valdivianus* that, 1) allocation patterns can be managed by the defoliation criteria applied and 2) allocation patterns are different in comparison to *L. perenne* (Donaghy and Fulkerson, 1998). The latter is based on that tiller population was not affected by the defoliation frequencies used; however, to reach further conclusions more research is needed.

3.5 Conclusions

Bromus valdivianus is a ‘six-leaf’ species, with three leaves growing simultaneously, and leaf senescence beginning at LS-4.25. *Bromus valdivianus* has a greater leaf area and tiller size, but a lower tiller number per plant, compared with *L. perenne*.

Shoot growth rate and total WSC levels indicated that LS-4 and LS-5 were the defoliation intervals that encouraged herbage mass accumulation.

Root growth rate was highly affected by defoliation frequency; LS-5 was the defoliation interval that most encouraged root growth rate.

Bromus valdivianus photosynthesis rate was not significantly different to that reported for other grasses (e.g. *L. perenne*) but its advantage, from the tiller point of view, was the higher leaf area, generating higher total photosynthesis per tiller.

Chapter 4: Experimental chapter

Soil - Plant - Atmosphere continuum: Different species traits and their effect on soil water content, plant growth and plant physiology in field conditions under monoculture and mixture swards

Summary

- Under the current climate change scenario, increasing the functional traits in a pasture may bring stability during water stress periods. This could be achieved by including species with tolerances to different environmental stresses. The botanical composition and herbage mass production in pastures, and physiological growth parameters of *L. perenne*, *T. repens*, *B. valdivianus* and *P. lanceolata* were determined as monocultures and mixtures across seasons.
- The treatments were: T1) *B. valdivianus*; T2) *L. perenne* + *T. repens*; T3) *P. lanceolata*; T4) *B. valdivianus* + *L. perenne* + *T. repens*; T5) *B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*. Herbage mass, photosynthesis, leaf water relations and soil water content at 10, 40 and 70 cm soil depth were analysed. The evaluations were performed seasonally for 2 years including three summer periods within.
- T5 showed to produce more herbage mass during the soil water restriction and T2 during waterlogging in comparison to the other treatments. T1 and T3 showed a higher uptake at 40 cm and T1 at 70 cm soil depth due to the high proportion of *B. valdivianus* and *P. lanceolata* and both species showed the lowest osmotic potentials and leaf water potential and higher net photosynthesis and stomatal conductance during water restriction periods than *L. perenne*. In T5, *P. lanceolata* was dominant in 5 seasons and, *L. perenne* co-dominant in 4 seasons.
- The inclusion of species that differ in their physiological and morphological traits (roots depth) confer a higher herbage mass production during water restriction periods. Higher tolerance to water restriction was shown by *B. valdivianus* and *P. lanceolata* due to deeper soil water extraction than *L. perenne*. *Lolium perenne* had higher herbage mass production during the spring and *P. lanceolata* during summer in T5.

4.1 Introduction

Ecosystem stability as a function of biodiversity has been widely studied since the 1950s and it is proposed that greater plant diversity builds ecosystems that are more resilient to environmental stress (Elton, 1958; Tilman et al., 1994; Tilman et al., 1998; Tilman, 1999; Loreau et al., 2000; Hooper and Duke, 2004; Tilman et al., 2006; Isbell et al., 2009; Tilman et al., 2014). The main factors behind this greater stability include an increase in plant traits inside the ecosystem (Hooper and Vitousek, 1997; Isbell et al., 2009) and the growth asynchrony between species (Tilman, 1999; Isbell et al., 2009; Zhang et al., 2018). Both factors are important concepts for building more sustainable and stable grazing systems, especially in light of the current global warming, for which has been predicted that droughts may become more frequent (Lehner et al., 2006) and the temperature variability may increase (Zhang et al., 2018). Any variation of the climatic variables, e.g. temperature and rainfall, will make plant production more uncertain (Tubiello et al., 2007), which will be a threat for rain-fed pastoral systems.

Lolium perenne is the most widely sown pasture grass in temperate pastoral systems (Amin and Thomas et al., 1996; Kemp et al., 2002), however, it is a species with a shallow root distribution between 0-20 cm (Evans, 1978; Crush et al., 2005). Ordóñez et al. (2018) reported that *L. perenne* had a greater water extraction between 0-10 cm soil depth, a reflection of the quantity of functional roots in that zone. At a greater depth *L. perenne* diminished its water extraction in comparison to a highly diverse pasture comprising by *L. perenne*, *B. valdivianus*, *D. glomerata*, *H. lanatus*, *T. repens*, other clovers, low quality grasses and broad leaf species. Similar findings for *L. perenne* were reported by Neal et al. (2012), indicating that the roots depth reached up to 150 cm of soil depth, but the water extraction was minor compared to other perennial grasses, e.g. *D. glomerata*. In a pastoral

ecosystem, the functional traits (e.g. root depth) of each individual species constitute a variable as important as the quantity of species in a pasture (Hooper and Vitousek, 1997; Isbell et al, 2009). The interaction between these functional traits can be a tool to provide sustainable management at a low diversity of species (Hooper and Vitousek, 1997; Petchey et al., 2004) and it may improve pasture productivity in the long term and during stress periods. Previous studies have highlighted the importance of species traits in the ecosystem, such as, the soil water dynamics through deeper root traits (Gan et al., 2012; Ordóñez et al., 2018) and soil water redistribution (Skinner et al., 2004; Skinner et al., 2008). Therefore, increasing plant functional traits in a pasture increases complexity in an ecosystem, improving its stability to the impact of environmental constraints, e.g. soil water restriction or waterlogging (Tilman et al., 1997; Petchey et al., 2004).

Zhang et al. (2018) stated that growth asynchrony between species during their development improves pasture stability to environmental constraints, thus the lack of asynchrony between species could negatively affect pasture production and persistence. Therefore, species asynchrony, along with species diversity with different functional traits (e.g. different root depth) are desired characteristics within a pasture, this can be evaluated through the different species growth rates across different seasons.

Considering the latter argument, the following species have dissimilar attributes: *L. perenne* is a species with a shallow root distribution (Shallow-rooted species; Crush et al., 2005; 2009) with a lower water extraction from soil layers deeper than 10 cm soil depth, compared to other grasses (Ordóñez et al., 2018), high growth rate during spring and winter, but a low growth rate in summer (Callow et al., 2003; Descalzi et al., 2019) due to low soil water content (Ordóñez et al., 2018; Descalzi et al., 2019); *Trifolium repens* is a legume with shallow-rooted distribution (Nichols et al., 2016), which has a

high growth rate during spring, but a low growth rate during summer and winter (Scheneiter et al., 2009). The lower growth rate in winter is due to its higher optimum temperature for growth (24 °C) in comparison to *L. perenne* (18 – 21 °C; Brock et al., 1989); *B. valdivianus* is a drought tolerant species (López et al., 2013), which may be due to its ability to access water from deep soil layers (Ordóñez et al., 2018). *Bromus valdivianus* has similar forage quality to *L. perenne* (Balocchi and López, 1996), a larger leaf area and higher tiller weight (López et al., 2013; Ordóñez et al., 2017), a greater number of live leaves per tiller but a lower tiller density (Ordóñez et al., 2017) and a high growth rate during summer, spring and autumn (Keim et al., 2015); *Plantago lanceolata*, a deep-rooted herb, up to 0.97 m length (Sanderson et al. 2003; Nie et al. 2008) and a high growth rate in late spring, summer and early autumn, but with a low growth rate during seasons with low temperatures (Pellissier, 2014). These four species have structural, physiological and functional differences; soil water extraction at different soil depths is correlated with functional roots distribution (Neal et al., 2012; Ordóñez et al., 2018), linked to physiological traits (Blum, 2009). When these species are grown together may perform asynchrony, improving pasture stability to environmental stress. However, this aspect needs to be investigated, along with the mechanisms that allow plant survival and growth under environmental stress, such as waterlogging or water restriction.

A relevant aspect to investigate is to what extent species with different functions are relevant to facilitate individual species survival within the same pasture. For example, do shallow-rooted species benefit, through increased water availability for example, by having deeper-rooted species as companions? Is there an effect on soil water dynamics due to different levels of functional diversity? The present research was designed to answer these questions using the deep-rooted species *B. valdivianus* and *P. lanceolata* together with shallow-rooted *L. perenne* and *T. repens* sown as mixed pasture.

The aim of the present study was to evaluate the effect of different levels of functional diversity, provided by its tolerance to different water stresses and its different growth strategies, on the changes in the plant community, plant physiology and soil water status, in pastures grown in the field and subjected to annual climatic fluctuation and grazing management. To accomplish this aim, leaf water relations, photosynthesis, stomatal conductance, soil water dynamics, herbage mass production and the pasture botanical composition changes across growing seasons were determined for *B. valdivianus* Phil, *L. perenne* L. and *P. lanceolata* L., grown at the establishment under monocultures and mixed swards.

4.2 Materials and Methods

4.2.1 Geographic location and weather description

The study was performed at Massey University's Number One Dairy Farm, Palmerston North (40°22'55.8"S 175°36'23.8"E). The soil was a Manawatu silty loam and was originated from a weathered fluvial recent soil (Hewitt 1998). The topsoil was a silty loam texture and the subsoil a sandy loam texture, very similar to the Manawatu fine sandy loam soil (Sanarath, 2003). The bulk density of the topsoil ranges between 1.1 and 1.2 Mg m³ and around to 1.3 Mg m³ over the 15 cm soil depth, and the low water storage capacity associated to the sandy textural properties in the subsoil (Senarath, 2003).

Regarding the field capacity (matric potential at 10 kPa), at 10 cm soil depth fluctuated between 42 – 43 % SWC; at 40 cm soil depth 32 - 33% SWC; and at 70 cm soil depth 13 – 14% SWC. The permanent wilting point (matric potential at 1500 kPa) at 10 cm soil depth ranged between 19 – 20% SWC; at 40 cm soil depth 19% SWC; and at 70 cm soil depth 7 – 8% SWC (Senarath, 2003).

The climate was temperate humid; the daily average air temperature, water atmospheric demand and the rainfall during the study are shown in Figure 4.1 and were extracted from a weather station located at 200 m from the experimental site (Agent number 21963; authorship to AgResearch).

The monthly rainfall deficit was determined based on the total monthly rainfall and the monthly mean potential evapotranspiration. The mean temperature was determined hourly and the average calculated using all the measurements between 0 and 23 hr.

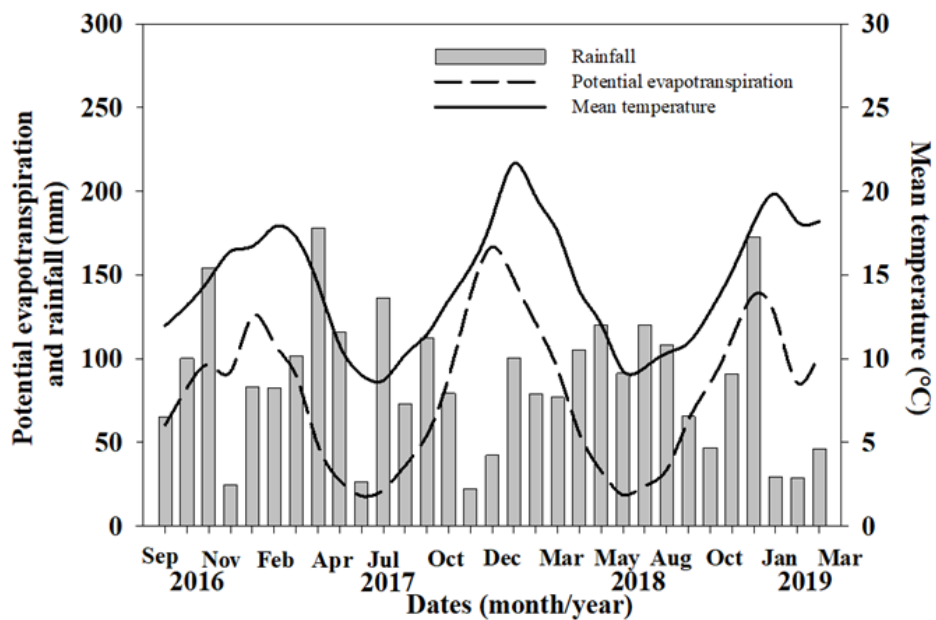


Figure 4.1 Rainfall, mean monthly air temperature and mean monthly potential evapotranspiration estimated with the Penman Monteith equation during the experimental period, between September 2016 and March 2019.

Rainfall, monthly mean temperature and monthly mean potential evapotranspiration for the evaluated period are shown in Figure 4.1. The total rainfall for the years 2017 and 2018 were 1,053 mm and 1,176 mm, respectively. The Penman-Monteith equation (Allen

et al., 1998) was used to observe the periods where the atmospheric water demand was higher than the rainfall.

4.2.2 Treatments description

The pastures were sown on the 20 October 2016 and the experiment commenced on 08 January 2017 and finalised by the end of March 2019. The experiment consisted in five types of pastures with 20 plots in total, established in 110 m² (10 x 11 meters) plots according to a randomised complete block design of 5 treatments x 4 blocks) The blocks were laid out perpendicular to the slope of the experimental site. All the pastures were sown after seedbed preparation, which consisted in ploughing and harrowing. Soil preparation was focus on the first 20 cm soil depth. After the sowing, the experimental site was rolled to increase the soil-seed contact. The species and sowing rate for each treatment were: T1) *B. valdivianus* (cv. Bareno; 30 kg ha⁻¹); T2) *L. perenne* (cv. Rohan; 25 kg ha⁻¹) + *T. repens* (cv. Weka; 3 kg ha⁻¹); T3) *P. lanceolata* (cv. Tonic; 14 kg ha⁻¹); T4) *B. valdivianus* (20 kg ha⁻¹) + *L. perenne* (12 kg ha⁻¹) + *T. repens* (3 kg ha⁻¹); T5) *B. valdivianus* (20 kg ha⁻¹) + *L. perenne* (12 kg ha⁻¹) + *T. repens* (3 kg ha⁻¹) + *P. lanceolata* (2 kg ha⁻¹). *Plantago lanceolata* cv. Tonic was chosen because, at that time, was the cultivar of *P. lanceolata* most used by farmers; *B. valdivianus* cultivars cv. Bareno is the only cultivar of this species available in market in New Zealand (Agriseeds, 2013); and *Lolium perenne* cv. Rohan, because is coming from a Spanish genetic line, which has attributes for water restriction tolerance and a high capacity to produce tillers, filling up the empty spaces between the sowing rows faster than the other *L. perenne* cultivars (Agriseeds, 2014). Sowing rates were based on industry recommendations for either monocultures or mixtures of the relevant species (Agricom, 2017).

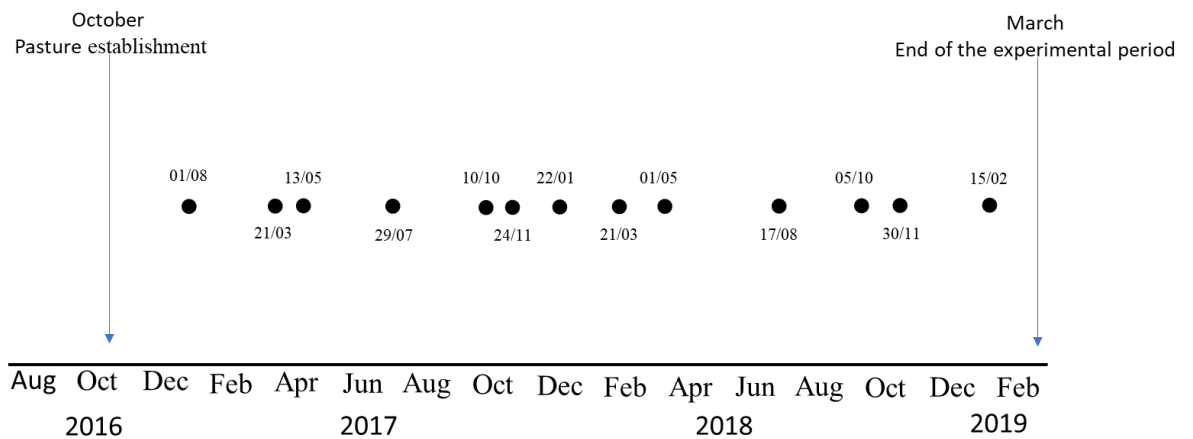


Figure 4.2 Grazing dates for the whole experimental period.

The grazing was performed to all treatments when *B. valdivianus* reached leaf stage 4.0. That meant a tiller with 6 live leaves, in which the 6th leaf had a leaf stage score of 0.25 (Ordóñez et al., 2017; Chapter 3). At the moment of the defoliation, *L. perenne* reached 3.5 leaf stage (Chapter 3) and *P. lanceolata* had over six leaves per plant. Each plot was grazed as a unit, being two blocks grazed at the same time. Each grazing was performed using 5 male sheep per plot, equivalent to a stocking density of 454 sheep ha⁻¹, with a grazing period of two days.

The grazing events performed during the experimental period are shown in Figure 4.2. Previous to each grazing event, there were taken into account; a) morpho-physiological development of *B. valdivianus* according to the defoliation criteria described in the Section 4.2.2, explained above; and b) the soil moisture conditions in order do not harm the pasture growth and the soil conditions as a consequence of the animal trampling.

4.2.3 Soil water content dynamics

In order to determine soil water uptake, especially during the stress periods, soil water content (SWC) was determined for all the treatments at three different soil depths: 10, 40 and 70 cm, using CS616 soil moisture sensors (sensor length: 30 cm; Campbell Scientific,

Logan, Utah, USA) connected to a CR1000 data logger (Campbell Scientific, Logan, Utah, USA) with a 16/32B multiplexer (Campbell Scientific, Logan, Utah, USA) installed. There were two soil moisture sensors at each soil depth. Sensors were installed at the 10 and 40 cm soil depths on the 11th October 2017, and at the 70 cm soil depth on the 29th October 2018.

In order to determine the severity of the soil water restriction matric potential sensors (model 229, Campbell Scientific, Logan, Utah, USA) were installed at the 10 and 40 cm soil depths on the 15th October 2018 and connected to the same data logger as the soil moisture sensors. As with the soil moisture sensors, two temperature and matric potential sensors were placed at each soil depth. Data was recorded every 30 min from installation date until the end of the study on the 30th March 2019.

The calibrations of the matric potential sensors were performed using 3 undisturbed soil samples according to the manual of these sensors (model 229, Campbell Scientific). The sensors were inserted and fixed into the undisturbed soil samples, then the samples were saturated from beneath for 48 h and then equilibrated at matric potential values of -1, -2, -3, -6 kPa in shelves with different height and all the samples were covered with plastic to avoid losses by evaporation. Then the samples were equilibrated at -15, -33, -50 and -1500 kPa (in pressure chambers). For each matric potential the sensors were connected to the datalogger when the soil samples reached the equilibrium (Campbell Scientific, Logan, Utah, USA). The soil matric potential was evaluated with a frequency of 10 minutes for one hour. Any matric potential was evaluated with a frequency of 10 minutes per 1 hour.

4.2.4 Leaf water potential and leaf osmotic potential

In order to determine the physiological response of *B. valdivianus*, *L. perenne* and *P. lanceolata* to the different soil water contents, leaf water potential and leaf osmotic potential were evaluated in all the treatments on the following dates: 10/03/2017; 17/03/2017; 22/11/2017; 16/01/2018; 26/04/2018; 01/08/2018; 28/09/2018; 22/11/2018; 28/01/2019. Both variables were measured simultaneously on the last fully expanded leaf, with the same leaf utilised to determine both variables. The plant material was collected before sunrise and at midday during the indicated dates.

Leaf water potential was evaluated with a Scholander pressure bomb (Soil Moisture Equipment Crop., Santa Barbara. CA). For osmotic potential, leaf samples were immersed into liquid nitrogen for 20 seconds to break the cellular wall. Immediately after that, the samples were stored in an insulated container with liquid nitrogen to maintain the temperature below 0°C until all samples had been collected. Samples were then taken to a freezer and stored at -80°C. To determine leaf osmotic potential, the samples were defrosted and immediately evaluated with a HR-33T Dew Point Microvoltmeter (Wescor, Logan, Utah, USA) using C-52 chambers (Wescor, Logan, Utah, USA) and following the methodology described by Turner et al. (1978). Chambers were calibrated before measurements started, with NaCl solutions at 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 molar as indicated by the Wescor C-52 chambers manual (Wright et al., 1983), in order to convert μV to bars.

4.2.5 Gas exchange

In order to determine the species performance under different weather conditions net photosynthesis (A_n), stomatal conductance (G_s), intercellular carbon concentration (C_i) and water use efficiency (WUE) were determined for *B. valdivianus*, *L. perenne* and *P.*

lanceolata by placing the last expanded leaves of two tillers per species for the grasses and one full healthy leaf of *P. lanceolata* inside the chamber. Evaluations were performed during the following dates: 10/03/2017; 17/03/2017; 22/11/2017; 26/04/2018; 01/08/2018; 08/08/2018; 05/10/2018; 28/01/2019. These variables were evaluated in the daily hour of highest atmospheric water demand, between 11:00 and 14:00. Therefore, the differences between species will reflect the root exploration in the soil and its water access. The gas exchange was evaluated using a LICOR-6400XT (LI-COR, Nebraska, USA) chamber, with a 6400-02B LED light source (LI-COR, Nebraska, USA). The photosynthetic photon flux density at the leaf surface was set at 1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, leaf temperature was stabilised to 25°C, the flow rate at 500 $\mu\text{mol s}^{-1}$ and the ambient CO₂ concentration of the incoming gas stream was set at 400 $\mu\text{mol s}^{-1}$.

$$\text{WUE} = \text{A}_n / \text{G}_s \quad (\text{eq. 4.1})$$

Where:

WUE = Water use efficiency ($\mu\text{mol m}^2 \text{ CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)

A_n = net photosynthesis ($\mu\text{mol m}^2 \text{ s}^{-1}$).

G_s = G_s is the stomatal conductance ($\mu\text{mol m}^2 \text{ s}^{-1}$).

Both, A_n and G_s, comes from the same evaluation, on the same leaf and at the same time.

4.2.6 Herbage mass and botanical composition

In order to determine the effect weather and defoliation by grazing, botanical composition was evaluated seasonally. In a temperate humid climate, each season, spring (1 September – 1 December), summer (1 December – 1 March), autumn (1 March – 1 June) and winter (1 of June – 1 September), is characterised by a particular weather.

The botanical composition sampling was done according to Radcliffe et al. (1968). For each evaluation event, two pasture samples of 0.1 m² quadrat per plot were taken once a season before a grazing event, cut down to ground level using a sheep shearing handpiece and a portable battery during the second month of each season, from summer (February), 2017 until summer (February) 2019. From this pasture sample all the species were manually separated and identified as plants of *B. valdivianus*, *L. perenne*, *T. repens*, *P. lanceolata*, broad leaf species, and other grasses, each species were placed in individual bags and oven dried at 70°C for two days to obtain dry weight. With the dry weight of the whole sample and the dry weight of each species or group of species within the whole sample, the percentage of participation in the botanical composition of the pasture was calculated.

$$\text{Species \%} = \text{DW}_{\text{species}} * 100 / \text{DW}_{\text{sample}} \quad (\text{eq. 4.2})$$

Where:

Species % = Participation, in percentage, of each species or groups of species within the whole pasture sample.

DW_{species} = Herbage mass (g DM) of each species or group of species indicated.

DW_{sample} = Herbage mass (g DM) of the whole pasture sample.

100 = Mathematical constant to transform the values to percentages.

The herbage mass was evaluated before and after grazing (Earle and McGowan 1979), two 0.1 m² samples per plot were cut down to ground level, dried at 70°C for two days, and weighed.

The accumulated herbage mass was determined as follow:

$$\sum_{i=1}^n (DW_{pre} - DW_{post}) = DW_{pre\ i} - DW_{post\ i} + DW_{pre\ 2} - DW_{post\ 2} + \dots + DW_{pre\ n} - DW_{post\ n}$$

(eq. 4.3)

Where:

Σ = Sum of dry weight (kg ha⁻¹) from the subtraction of DW_{pre} and DW_{post} of each grazing.

n = Total number of grazing across the whole experimental period.

i = Initial grazing.

DW_{pre} = Dry weight before the grazing (kg ha⁻¹)

DW_{post} = Dry weight after the grazing (kg ha⁻¹)

To determine the growth rates of each season, the average of growth rates between several grazing events within the same seasons was calculated.

The growth rates were determined as follow:

$$\text{Growth rate} = DW_{pre\ n} - DW_{post\ n-1} \text{ (eq. 4.4)}$$

Where:

Growth rate = Accumulated herbage mass between the post grazing herbage dry weight from the previous grazing event (n-1) and the pre grazing herbage dry weight of the following grazing event (n).

n = any grazing event.

n -1 = previous grazing event in relation to grazing event (n).

$DW_{post\ n-1}$ = Herbage dry weight of the post grazing from the previous grazing event in relation to grazing event (n).

$DW_{pre\ n}$ = Herbage dry weight of the pre grazing evaluation from grazing event (n).

4.2.7 Experimental design and statistical analysis

The experiment was laid out according to a randomised complete block design (5 treatments x 4 blocks). The normality of the data was evaluated using the Kolmogorov-Smirnov test ($p \leq 0.05$). Analysis of variance (ANOVA) was applied to the normally distributed data. Fisher's least significance difference test (LSD) ($p \leq 0.05$) was used to separate treatment means (Steel et al. 1997). When data were not normally distributed, mathematical functions were applied to normalise them. This was the case for stomatal conductance (normalised with root square function), leaf osmotic potential (normalised with cube-root) and water use efficiency (normalised with natural logarithm). When data transformation was not able to confer normality to the data, such as intercellular carbon concentration (C_i), or if the objective was to show the population distribution of the data, such as water use efficiency (WUE), non-parametric statistics were applied (box diagrams, mean, median, percentiles, and standard deviation), and the Kruskal-Wallis's test ($p \leq 0.05$) was used to compare the treatment mean.

4.3 Results

4.3. Soil water dynamics

The effect of plant water uptake and evaporation is reflected on the soil water dynamics across all the evaluation period (Figure 4.3; low SWC reflect a high water uptake) where two variables changed the soil water dynamics and showed opposite trends, are indicated as follows; 1) the effect of water atmospheric demand diminished with soil depth and 2)

the SWC differences between treatments increased with soil depth may be due to the differences in root depth distribution of the sown species.

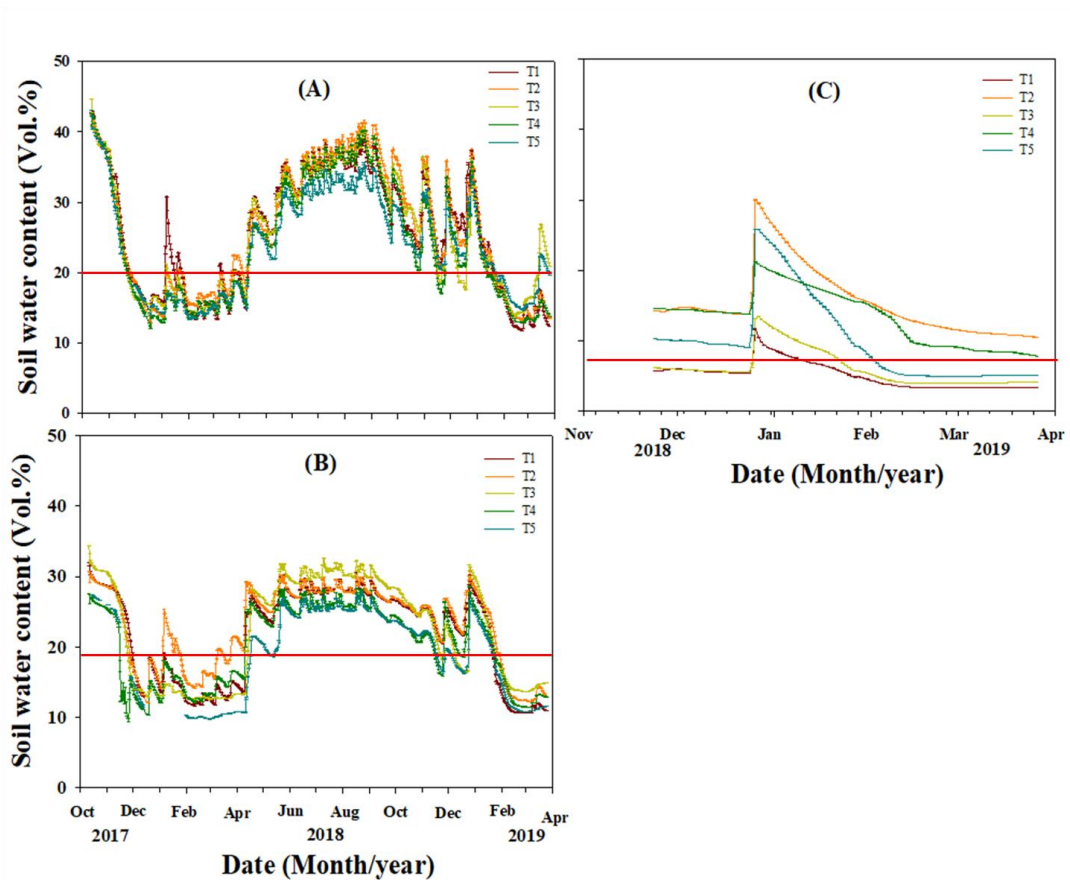


Figure 4.3 Soil water content for all the treatments at three depths, at 10 (A) cm and 40 cm (B) between October 2017 and March 2019; and at 70 cm (C) soil depth between October 2018 and April 2019. T1 (*B. valdivianus*); T2 (*L. perenne* + *T. repens*); T3 (*P. lanceolata*); T4 (*B. valdivianus* + *L. perenne* + *T. repens*); T5 (*B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*). Bars indicate standard error of the mean (n= 48). Red lines indicate the estimated permanent wilting point at 10 cm (20% SWC; A) and at 40 cm soil depth (19% SWC; B), and at 70 cm soil depth (8% SWC; C) determined according to Senerath (2003).

At 10 cm soil depth, SWC was difficult to determine, due to the variables involved, i.e. root abundance and atmospheric demand, resulting in SWC being unstable over time.

However, the treatments with lower SWC at 10 cm soil depth were T1, T4 and T5 (Figure 4.3A) during almost all year, especially during the water restrictions periods. On the other hand, the treatments T2 and T3 tend to have a higher SWC during almost all the evaluation period, with some exceptions.

At 40 cm soil depth the soil water dynamics were more easily to identify the treatments differences, registering that T1 and T5 had the lowest SWC values during almost soil water restriction. T1 on the other hand during non-water restriction periods showed similar values than T2 and T3. During the soil water restriction periods T3 showed a higher water extraction compared to T2 and T4, but during autumn, winter and early spring is one of the treatments with the higher SWC (Figure 4.3B).

At 70 cm soil depth there were differences over the last water deficit period (Figure 4.3C), such that treatment T1, followed by T3, showed the lowest values. T5 and T4 experimented significantly higher water extraction than T2. The most probable explanation is the differences in the functional roots distribution, for which the root number and density that reached that soil depth have a significant influence on SWC. For example, T3 (*P. lanceolata*) could have had a deeper root system but its root density was not enough to overcome the water extraction done by *B. valdivianus* at that soil depth.

The calibration of the 229 sensors shown that the permanent wilting point at 10 cm soil depth was about 20% SWC and at 40 cm soil depth about 19% SWC, results that agreed with Senerath (2003). For the subsoil layer at 70 cm soil depth the permanent wilting point was based on the data presented by Senerath (2003).

4.3.2 Daily variation in soil water content during soil water restriction

Significant SWC daily variation at 10 cm soil depth ($p \leq 0.001$; Table 4.1) were assessed and registered every 30 minutes. The pattern of a daily SWC variation commenced when the SWC was below 16%, or when it exceeded the 2000 kPa of soil matric potential (Figure 4.3). This situation occurred in three periods across all the period of evaluation and were the following: 28/12/2017 until 04/01/2018; 02/02/2018 until 06/03/2018; 09/02/2019 until 09/03/2019. This pattern constantly reached its minimum daily SWC at 08:00, while the maximum was detected at 17:00 ($p \leq 0.05$).

Table 4.1 Differences in soil water content, at 08:00 (minimum) and 17:00 (maximum) for the three periods of water deficit (below 16% of soil water content) for all the treatments.

hour	First period						SEM first period				
	T1	T2	T3	T4	T5	p- value	T1	T2	T3	T4	T5
08:00	16.497b	15.421a	15.809b	14.285a	15.685b	≤ 0.05	0.203	0.322	0.104	0.269	0.252
17:00	17.053a	15.869a	16.397a	14.704a	16.054a		0.208	0.330	0.119	0.263	0.265
Second period							SEM second period				
08:00	14.470a	16.059b	14.791b	14.685b	14.114b	≤ 0.05	0.161	0.124	0.081	0.156	0.117
17:00	14.739a	16.539a	15.009a	15.089a	14.517a		0.157	0.125	0.079	0.151	0.113
Third period							SEM third period				
08:00	13.550a	13.879a	16.070b	13.120b	15.328b	≤ 0.05	0.371	0.169	0.217	0.048	0.123
17:00	13.913a	14.185a	16.649a	13.501a	15.629a		0.336	0.171	0.183	0.064	0.130

*Note: Data analysed was considering the SWC readings between 30 minutes. SEM is the standard error of the mean, for the first period $n = 8$; second period $n = 32$; third period $n = 29$. First period between 28/12/2017 - 04/01/2018; second period between 02/02/2018 - 06/03/2018; third period between 09/02/2019 - 09/03/2019. T1 (*B. valdivianus*); T2 (*L. perenne* + *T. repens*); T3 (*P. lanceolata*); T4 (*B. valdivianus* + *L. perenne* + *T. repens*); T5 (*B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*).*

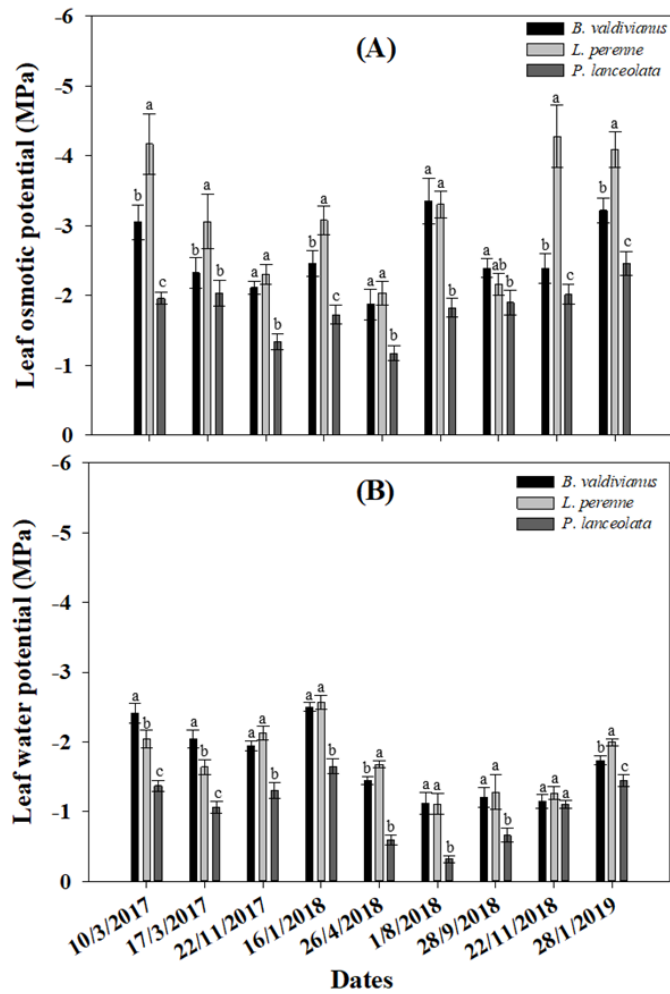


Figure 4.4 Leaf osmotic potential for *B. valdivianus*, *L. perenne* and *P. lanceolata* during all the periods of evaluation (A). Leaf water potential for *B. valdivianus*, *L. perenne* and *P. lanceolata* (B). Bars indicate standard error of the mean (n= 3).

The daily SWC pattern fluctuation is presented in Table 4.1. The fluctuation occurred without any addition of water by any source and was between (mean \pm sem) 0.12% SWC ($\pm 0.054\%$) and 0.58% SWC ($\pm 0.068\%$), reaching the daily maximum at 17:00 and the daily minimum at 08:00. T3 showed differences in all three periods, exhibiting the largest differences in daily SWC variation during the first period, registering a SWC change of 0.59%. The largest variation for T2 was registered during the second period with a SWC change of 0.58%. However, T2 did not show statistical differences for daily variation in

SWC during either the first or third periods named before. The largest daily variation in SWC during the third period was for T3, with a value of 0.58%.

4.3.3 Leaf osmotic potential and leaf water potential

Statistical differences were found for leaf osmotic potential ($p \leq 0.001$) and leaf water potential ($p \leq 0.001$) for *B. valdivianus*, *L. perenne* and *P. lanceolata*, with a significant interaction between species x date for leaf water potential ($p \leq 0.05$) and for leaf osmotic potential ($p \leq 0.01$). No statistical differences were found when the same species was compared in different treatments ($p > 0.05$).

Lolium perenne had higher osmotic potential values during the soil water restriction periods (Figure 4.4A) compared to *B. valdivianus* and *P. lanceolata*, the situation described as follows: *L. perenne* showed a higher leaf osmotic potential in 5 over 9 measurements, the maximum value was accomplished by *L. perenne* as well, reaching over -4.0 MPa in three dates (mean \pm sem): 10/03/2017 (-4.17 ± 0.85 MPa), 22/11/2018 (-4.27 ± 0.45 MPa) and 30/01/2019 (-4.09 ± 0.26 MPa). The maximum values for *B. valdivianus* were over -3.0 MPa in three dates: 10/03/2017 (-3.05 ± 0.25 MPa), 01/08/2018 (-3.17 ± 0.33 MPa) and 30/01/2019 (-3.22 ± 0.28 MPa). The maximum values for *P. lanceolata* were over -2.0 MPa in three dates: 17/03/2017 (-2.01 ± 0.14 MPa), 22/11/2018 (-2.04 ± 0.18 MPa) and 30/01/2018 (-2.50 ± 0.26 MPa).

4.3.4 Gas exchange: net photosynthesis, stomatal conductance, intercellular carbon concentration and water use efficiency

Statistical differences for net photosynthesis (A_n ; $p \leq 0.001$) and stomatal conductance (G_s ; $p \leq 0.001$), and water use efficiency (WUE; $p \leq 0.05$), and no differences for intercellular carbon concentration (C_i ; $p > 0.05$) were assessed. Significant interactions were found

between species x date for A_n ($p \leq 0.001$) and G_s ($p \leq 0.001$). No interactions were found between species x date for C_i ($p > 0.05$) and WUE ($p > 0.05$). During the periods without soil water restriction, A_n reached its highest value. *Lolium perenne* had the highest A_n , with (mean \pm sem) $30.3 \mu\text{mol m}^2 \text{s}^{-1}$ (± 3.7) on 17/03/2017 and $26.6 \mu\text{mol m}^2 \text{s}^{-1}$ (± 1.7) on 01/08/2018 (Figure 4.5A). Also *P. lanceolata* and *B. valdivianus* reached higher A_n compared to *L. perenne* for the following dates: 22/11/2017, 05/10/2018 and 30/01/2019.

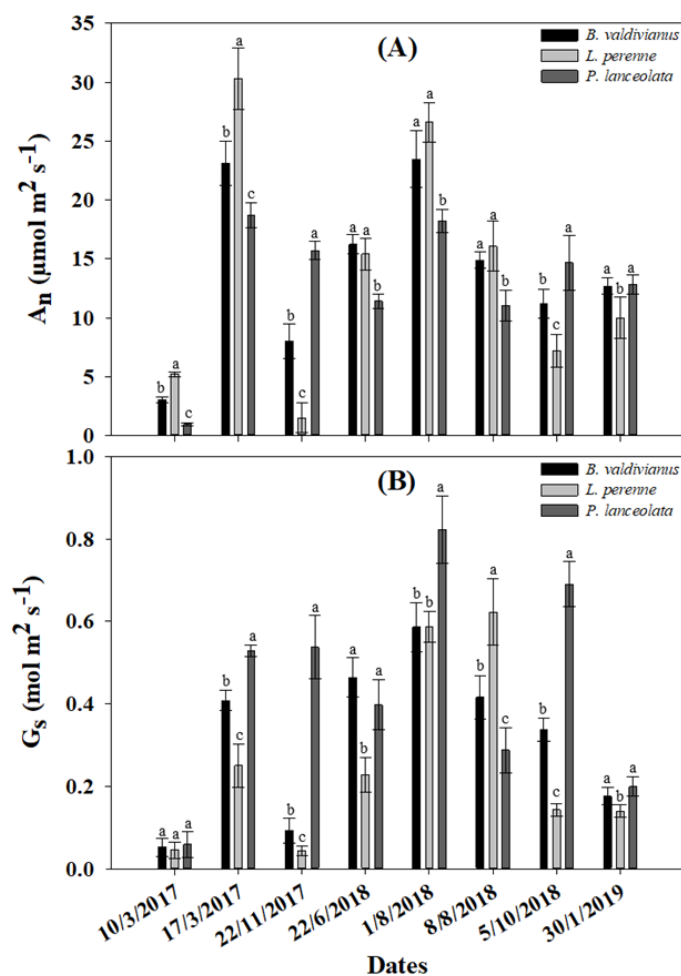


Figure 4.5 Gas exchange for net photosynthesis (A_n) for *B. valdivianus*, *L. perenne* and *P. lanceolata* during the period of evaluation (A). Stomatal conductivity (G_s) for *B. valdivianus*, *L. perenne* and *P. lanceolata* during the period of evaluation (B). Bars indicate standard error of the mean (n= 3).

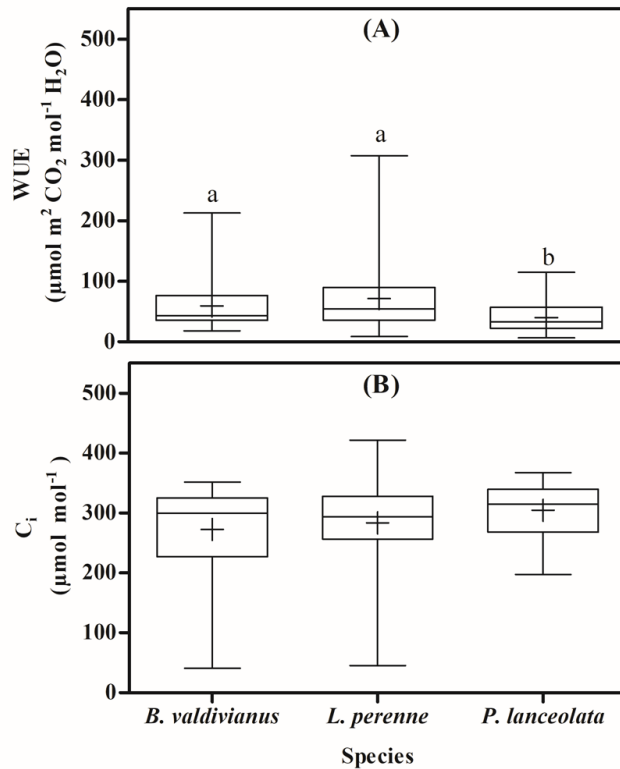


Figure 4.6 Water use efficiency (An/Gs; WUE) (A) and intercellular carbon concentration (C_i) (B) for *B. valdivianus*, *L. perenne* and *P. lanceolata* during all period of evaluation. Lowercase letters indicate statistically differences. + indicates the mean, bars and boxes the distribution of the population (quartiles) (n=72).

For G_s (Figure 4.5B), *P. lanceolata* showed higher G_s than *L. perenne* in 6 over 8 evaluations and higher than *B. valdivianus* in 4 over 8 evaluations. The maximum value of (mean \pm sem) $0.822 \text{ mol m}^{-2} \text{ s}^{-1}$ (± 0.081) was reached by *P. lanceolata* during 01/08/2018. The highest value for *B. valdivianus* was (mean \pm sem) $0.585 \text{ mol m}^{-2} \text{ s}^{-1}$ (± 0.060) on 01/08/2018 and for *L. perenne* (mean \pm sem) $0.622 \text{ mol m}^{-2} \text{ s}^{-1}$ (± 0.0082) on 08/08/2018. Generally, especially during soil water restrictions periods, *P. lanceolata* and *B. valdivianus* had higher G_s than *L. perenne*.

Significant differences ($p \leq 0.05$) in WUE were measured between the species (Figure 4.6A). *Lolium perenne* and *B. valdivianus* recorded a higher median, $54.26 \mu\text{mol m}^2 \text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ and $43.48 \text{ m}^2 \text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$, respectively, in contrast to *P. lanceolata* with $33.02 \text{ m}^2 \text{CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$.

For C_i , no significant differences ($p > 0.05$) were found, with a median for *B. valdivianus*, *L. perenne*, and *P. lanceolata* of $299.9 \mu\text{mol mol}^{-1}$, $293.7 \mu\text{mol mol}^{-1}$ and $314.8 \mu\text{mol mol}^{-1}$, respectively (Figure 4.6B).

4.3.5 Herbage mass and seasonal botanical composition

In table 4.2 statistical differences for growth rate ($p \leq 0.05$) between Autumn 2017 and summer 2019 were found and with significant interaction between treatments x date ($p \leq 0.001$) for growth rate. In table 4.3 regarding to accumulated herbage mass, no differences were found ($p > 0.05$) nor interaction between treatments x year ($p > 0.05$). However, differences between years were founded ($p \leq 0.01$), where the second year was statistically higher than the first year of growth (Table 4.2).

The seasonal growth rates for each pasture treatment showed that T5 had higher growth rates in 5 out of 8 seasons: T5 = T3 (autumn 2017); T5 = T2 = T4 = T1 (spring 2017); T5 > T1, T2, T3, T4 (summer 2018); T5 = T1 = T2 = T4 (spring 2018); T5 > T1, T2, T3, T4 (summer 2019). During the two winter periods (2017 and 2018), T2 showed higher growth rates as shown in Table 4.9: T2 = T1 = T3 (winter 2017); T2 > T1, T3, T4, T5 (winter 2018).

There were significant differences ($p \leq 0.05$) in the botanical composition between and within the treatments across seasons (Figure 4.7). At the beginning of the study, T1 (Figure 4.7A) comprised (mean \pm sem) $73.2\% (\pm 6.1)$ *B. valdivianus*, which decreased to

a minimum of 55% (± 5.7) by the end of winter 2017. This decrease in *B. valdivianus* was associated to an increase of unsown *L. perenne*, which was not present at the beginning of the evaluation in T1, but during the spring 2017 *L. perenne* reached 24.6% (± 3.7) of T1 dry matter (DM) production.

Table 4.2 Seasonal growth rate of the herbage mass (kg DM ha⁻¹ day⁻¹) for the two years of evaluation (between autumn 2017 and summer 2019).

Treatments	2017				2018			2019
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
T1	40.8 b	18.8 ab	60.7 ab	44.1 b	60.2 a	17.1 d	120.6 a	73.3 b
T2	41.4 b	26.9 a	73.4 a	44.2 b	48.5 b	27.1 a	99.5 a	42.9 d
T3	58.4 a	22.1 ab	53.8 b	33.9 b	35.9 c	20.9 bc	85.6 b	64.2 c
T4	41.5 b	14.4 b	66.0 ab	36.9 b	60.7 a	18.3 cd	107.5 ab	47.5 d
T5	63.8 a	13.0 b	71.9 a	60.4 a	46.8 bc	22.4 b	89.9 ab	91.4 a
p-value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
SEM								
T1	5.4	4.2	12.3	6.6	7.9	1.4	14.4	3.4
T2	6.5	6.0	11.1	3.2	3.4	2.1	6.0	3.0
T3	6.3	8.5	6.7	9.1	5.8	2.2	5.6	3.0
T4	6.8	4.2	12.4	5.5	4.9	2.3	17.3	7.2
T5	10.0	2.2	6.5	6.4	5.6	1.1	15.2	14.3

Note: Lowercase letters indicate statistical differences between treatments ($n = 8$) ($p \leq 0.001$). SEM is standard error of the mean ($n = 4$). T1 (*B. valdivianus*); T2 (*L. perenne* + *T. repens*); T3 (*P. lanceolata*); T4 (*B. valdivianus* + *L. perenne* + *T. repens*); T5 (*B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*).

T2 botanical composition (Figure 4.7B) comprised (mean \pm sem) 76.3% (± 0.1) *L. perenne*. After that, consistently increased to a peak of 95% ($\pm 1.7\%$) in autumn 2018. By summer 2019, *L. perenne* declined in T2 to 76.9% ($\pm 1.4\%$). *Trifolium repens* reached

around 20% ($\pm 0.7\%$) during Autumn 2017, there on onwards showed sharp decline with no significant participation within the pasture production.

Table 4.3 Accumulated herbage mass (kg DM ha⁻¹ year⁻¹) for the two years of evaluation (between summer 2017 and summer 2019) of all the treatments.

Treatments	Year 1	Year 2	SEM Year 1	SEM Year 2
T1	13,227	18,741	1,327	940
T2	15,675	16,832	2,509	392
T3	14,024	15,697	1,941	747
T4	13,445	17,373	953	605
T5	17,264	18,981	1,317	399
p-value	>0.05	>0.05		
Year average	14,727b	17,525a	852	426
p-value	≤ 0.01			

Note: SEM is the standard error of the mean ($n = 4$). T1 (*B. valdivianus*); (B) T2 (*L. perenne* + *T. repens*); (C) T3 (*P. lanceolata*); (D) T4 (*B. valdivianus* + *L. perenne* + *T. repens*); (E) T5 (*B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*).

In T3, *Plantago lanceolata* (Figure 4.7C) contributed around (mean \pm sem) 90% ($\pm 4.2\%$) of pasture DM composition for the first 18 months of the field evaluation (summer 2017 to winter 2018), and then declined towards the end of the study to 79.3% ($\pm 4.8\%$; spring 2018 and summer 2019). This decline was associated with an increase in *T. repens* from 1.1% ($\pm 0.8\%$) in winter 2018 to 14.3% ($\pm 3.6\%$) in summer 2019.

Despite that all three sown species in the T4 pasture (Figure 4.7D) started during summer 2017 with a high contribution of *L. perenne* (mean \pm sem) ($39.6 \pm 6.1\%$), *T. repens* ($30.9 \pm 8.5\%$) and *B. valdivianus* ($22.8 \pm 2.6\%$), by the spring 2017 *L. perenne* was dominating ($81.8 \pm 4.7\%$) the pasture, with *T. repens* being 13% (± 5.4) and *B. valdivianus*

3.4% (± 1.1). By the end of the study period, *B. valdivianus* increased to 30.7% (± 5.0), *L. perenne* dropped to 47.1% (± 9.7) and *T. repens* 5.2% (± 1.4), of pasture DM.

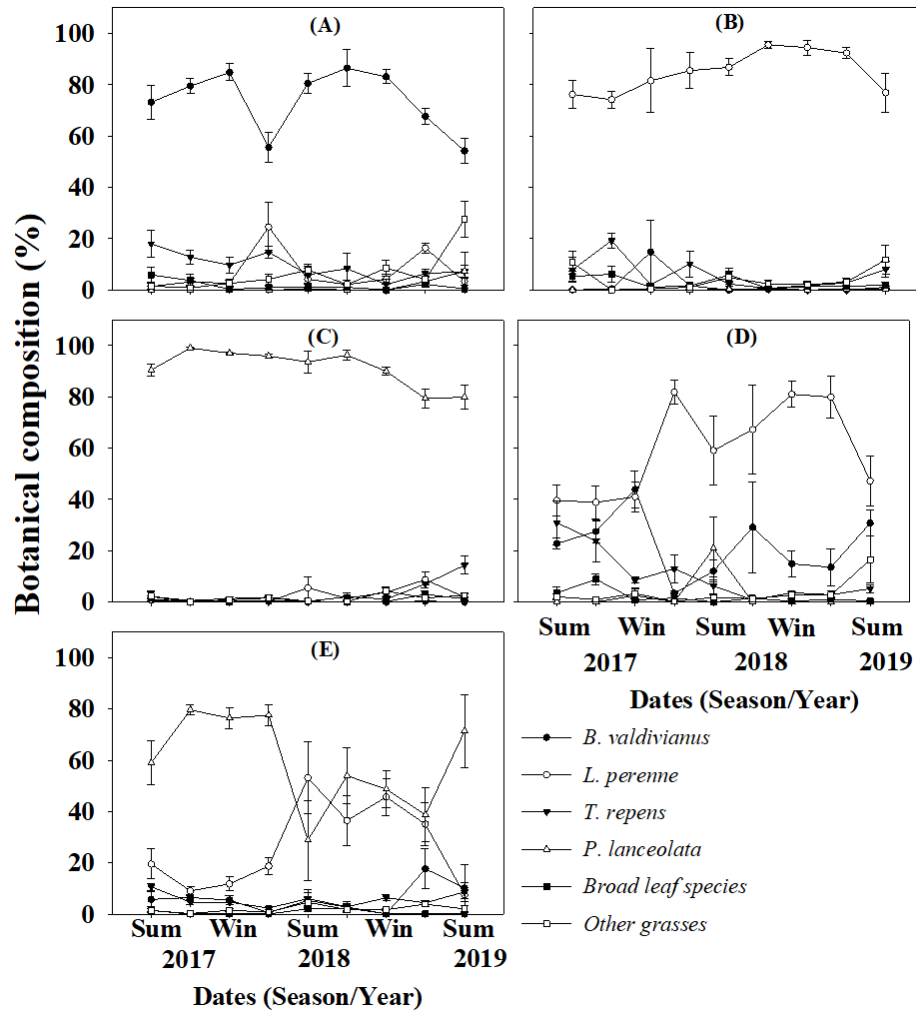


Figure 4.7 Botanical composition (% of dry matter); between summer 2017 and summer 2019. T1 (*B. valdivianus*) (A); T2 (*L. perenne* + *T. repens*) (B); T3 (*P. lanceolata*) (C); T4 (*B. valdivianus* + *L. perenne* + *T. repens*) (D); T5 (*B. valdivianus* + *L. perenne* + *T. repens* + *P. lanceolata*) (E). Bars indicate standard error of the mean (n= 4).

In T5, the pasture comprising all 4 sown species, *P. lanceolata* dominated the composition ($59.0 \pm 8.5\%$), over *L. perenne* ($19.5 \pm 5.6\%$), *T. repens* ($10.6 \pm 1.4\%$) and

B. valdivianus ($5.7 \pm 2.9\%$) until summer 2018, when the composition of *P. lanceolata* declined to $28.1\% (\pm 15.6)$ the composition of *L. perenne* increased to $51.3\% (\pm 14.1)$. At the end of the study (summer 2019), *P. lanceolata* again dominated the pasture with $71.3\% (\pm 14.3)$, while *L. perenne* declined until $7.5\% (\pm 1.5)$. On the other hand, *T. repens* and *B. valdivianus* showed a low contribution in the botanical composition with $8.7\% (\pm 1.1)$ and $10.2\% (\pm 4.5)$, respectively.

4.4 Discussion

4.4.1 Soil - plant - atmosphere continuum

Ecosystem stability is driven by functional diversity (Hooper and Vitousek, 1997), growth asynchrony,overyielding and portfolio effect (Tilman, 1999; Isbell, 2009; Loreau, 2010; Zhang et al., 2018). Asynchrony are associated with plant physiological processes, due to niche differentiation, temporal differentiation (e.g. different moments of growth and therefore different moments resources uptake) and space differentiation (e.g. different soil depth water uptake: Hooper and Duke, 2004). This is all reflected in the pasture botanical composition, seasonal growth and water uptake of the different species in a pastoral ecosystem. In climates with water restriction, water uptake in the deep soil layers (niche differentiation) (Hooper and Duke, 2004) would lead to an increase in G_s and A_n , increasing the production across the water restriction periods (Blum, 2009).

Increasing osmotic adjustments in plants is an adaptive response to water restriction (Blum, 2005; 2009; 2017), enabling the maintenance of cellular turgor, which allows the continuation of stomatal conductance under leaf water deficit (Sellin et al., 2001) and improves the water uptake at high soil matric potentials (Chimenti et al., 2006).

In other studies *L. perenne* reached values between a range of -1.6 to -6.0 MPa of osmotic potential (Thomas, 1987; Amin and Thomas, 1996, Volaire et al., 1998, He, 2016, Cyriac et al., 2017), meanwhile across this experiment the values of *L. perenne* reached a top of -4.2 MPa. This raises the question, of whether *L. perenne*'s greater capacity to increase its osmotic potential as an adaptation to soil water restriction is driven by genetics, or due to a response to its superficial water uptake capacity (between 0-10 cm; Crush et al., 2005; Ordóñez et al., 2018)? Either way, this mechanism allows it to enhance soil water uptake from soil water stored at a high matric potential at that depth (-6.0 MPa is four times the permanent wilting point), water that is used by the plant during transpiration (Chimenti et al., 2006; Blum, 2009). On the other hand, *B. valdivianus* and *P. lanceolata* registered a lower osmotic potential, and for *P. lanceolata*, a lower leaf water potential as well. This showed that both species were less stressed by the soil water restriction probably due to a higher water availability, due to deeper soil exploration than *L. perenne* (Nie et al., 2008; Neal et al., 2012, López et al., 2013; Ordóñez et al., 2018).

In the present study, *L. perenne* and *B. valdivianus* had similar values for A_n and the differences presented related to A_n and G_s indicate the response to the weather conditions i.e. soil water restriction or waterlogging. *Plantago lanceolata* was the species with the highest A_n and G_s during the soil water restriction periods followed by *B. valdivianus*, which along with a higher water uptake at deep soil layers (up to 70 cm), as a consequence of a deeper root distribution. High G_s values for *P. lanceolata* have been reported also by Dubbert et al. (2017). These results were positively related to the leaf water relations and soil water uptake. Even though, *P. lanceolata* had higher A_n and G_s and lower WUE, could be interacting with a lower stomata control, this assumption is done by the data collected and the morphological conditions of the plant during the sampling under water restrictions periods. Probably under heavy water restriction periods, if *P. lanceolata* do

not have access to enough water for its survival, its abundance would heavily decrease in time due its hypothetical lower stomata control.

Blum (2009) discussed whether WUE was the correct parameter to evaluate species and cultivars under conditions of limited water supply. The argument behind is that WUE, as a measure, does not comprehensively consider soil water uptake. The continuation of biomass production under a water restriction can be accomplished by maximising the water uptake from the soil (effectiveness of water use), such as osmotic adjustment (Blum, 2005; Chimenti et al., 2006) and how deep a root system is (Kobata et al., 1996; Pinheiro et al., 2005; Kirkegaard et al., 2007) as the strategy is to use the water stored in the soil, creating a water flows from the soil towards the plant due to transpiration mechanism and not allowing the water be loss by evaporation. Therefore, a higher G_s and a greater CO_2 fixation (A_n) improve the effective use of water (EUW) opposite to WUE, which increase as G_s and A_n diminish (Blum, 2009).

The latter could be the case of *P. lanceolata* (in T3 and T5), a species with low WUE, a higher soil water uptake than T2 (*L. perenne*; at 40 and 70 cm soil depth) and T1 (*B. valdivianus*; at 40 cm soil depth) and in T5 that was mainly dominated by *P. lanceolata*, had a greater performance during water restriction periods than T3 (*P. lanceolata* monoculture). The co-dominance between *L. perenne* and *P. lanceolata* in T5 showed how both species are good competitors complementing well with each other.

The results of the present study indicate that *P. lanceolata* had the lowest WUE but also a mayor EUW compared to *L. perenne* due to its greater A_n , G_s and water uptake during soil water restriction. On the other hand, *B. valdivianus* displayed a similar WUE than *L. perenne*, it also extracted more soil water at deeper depths, which resulted in a higher

EUW. The results obtained for *B. valdivianus* relating to WUE and EUW indicate that there are good opportunities for genetic improvement in both aspects.

The present study unfolded how *L. perenne*, *B. valdivianus* and *P. lanceolata* differ in their seasonal growth and their physiological behaviour during the seasons. Across periods of soil water restriction, the pasture dominated by *B. valdivianus* and *P. lanceolata*, grew faster than the ones dominated by *L. perenne*. *Plantago lanceolata*, in T5, was able to co-dominate the pasture botanical composition together with *L. perenne*, as measured during the summer of 2017 and 2019. Besides in T5, the higher growth rates during the water restriction period supports Hooper and Vitousek (1997) statement, that stability is achievable at low diversity but with species with different functional traits. However, *B. valdivianus* (T1) was more effective than *P. lanceolata* (T3 and T5) taking up the water at 70 cm soil depth. These results can be interpreted as: 1) *B. valdivianus* effectively had a deeper root system than *P. lanceolata*; or 2) For this experiment, the quantity of functional roots of *B. valdivianus* reaching 70 cm soil depth was higher than *P. lanceolata*, which does not mean that *B. valdivianus* has a deeper root system.

Bromus valdivianus strategy is to generate a large number of leaves alive per tiller, a large leaf area per tiller and a high tiller size, and as a trade-off, few tillers per plant (Chapter 3). Moreover, it is proposed that *B. valdivianus*, due to its greater leaf area (Chapter 3), similar A_n , and greater water uptake at 40 and 70 soil cm, allocates more photo-assimilates to the root system than *L. perenne*.

In relation to *B. valdivianus* EUW, results from the present study strongly suggest that *B. valdivianus*, from the data collected in relation to the treatment T1, had a higher soil water extraction at deep soil horizons, as it was measured up to 70 cm soil depth, being this an indication that *B. valdivianus* root system is deeper than *L. perenne* and possibly deeper

than *P. lanceolata*. Furthermore, roots lifespan determine the root growth period and how much resources are received to keep on growing, extending the time for the soil exploration and resources uptake. These issues remain not known for *B. valdivianus* and *P. lanceolata*.

Lolium perenne root lifespan was reported as 15 weeks, less than *Arrhenatherum elatius* (around 40 weeks), *Molinia caerulea* (over 50 weeks) and *Nordus stricta* (over 55 weeks) as indicated by Van Der Krift and Berendse (2002). Watson et al. (2000) showed how climate affected the roots survival for *L. perenne*. Two locations were evaluated (sites in UK and Italy). The trial performed in Italy showed that *L. perenne* lost 90% of its roots after 42 days, while root loss in the UK was 50% after 42 days. However, after 15 weeks in the UK trial, *L. perenne* lost 70% of its roots. This evidence may explain why *L. perenne* has a shallow root distribution: most of the root system does not remain alive long enough to reach the deeper soil layers, being a minimum root percentage from the total root biomass, the one that grows deep in the soil, explaining the low water uptake of *L. perenne* reported by Neal et al. (2012).

In relation to waterlogging, in August 2018, the SWC was around 36% which was close to saturation (around 43% SWC). Therefore, the soil had at about 7% of oxygen in the first 10 cm of soil depth, with a maximum environmental temperature at midday of 14 °C. Under these conditions, *L. perenne* G_s was significantly higher than both *P. lanceolata* and *B. valdivianus*, indicating *L. perenne* tolerance of waterlogging due to the presence of aerenchyma in its root system (Naceur et al., 2003). The lower *B. valdivianus* and *P. lanceolata* G_s during that time may be a sign of waterlogging sensitivity (Ploschuk et al., 2017; McFarlane et al., 2003), which could mean, low tolerance to low oxygen conditions (Drew, 1997). This argument is supported by the low *B. valdivianus* (T1 Table

4.3) growth rate during winter and Stewart (1996) statement about the low tolerance of *B. valdivianus* towards waterlogging conditions.

It is relevant to consider that *B. valdivianus* originated from an allophanic (Andisols) soils region, which are flat, deep and well-drained soils with medium texture (López et al., 1997), therefore *B. valdivianus* development always have been over soils with a great air capacity (Dörner et al., 2013).

Plantago lanceolata winter low growth rates could be also related to its temperature requirements, which are higher than those required by grasses (Niu et al., 2006; Pellissier et al., 2014).

4.4.2 Interaction between botanical composition and soil water dynamics

Soil water content is the outcome of several processes, such as rainfall, evaporation, water infiltration and plant water uptake (Simunek et al., 2003). The most relevant parameters during soil water restriction that define soil water content are the evaporation (French and Schultz, 1984; Siddique et al., 1990) and soil water uptake by plants (Simunek et al., 2003; Ordóñez et al., 2018), therefore the pasture water uptake is dynamic across seasons, lined up with the successional changes of the pasture botanical composition, as indicated by Dörner et al. (2013) and Ordóñez et al. (2018). Therefore, SWC has a close relationship with species root traits, functional roots, roots density and stomatal enclosure of the species (Blum, 2009).

Species succession has been defined as a change of species within a community, being related to abundance and spatial pattern distribution (Frankland, 1998). Disturbance and environmental stress may modify the botanical composition of plant communities. In a pasture, stress and/or disturbance may result in bare ground areas (Pickett and White,

1985), allowing other species to colonise these gaps, changing the botanical composition and the structure of the plant community (Crawley and May, 1987). Increasing bare ground spaces in a pasture and the colonisation by other species also increases (Marriot et al., 1997). Therefore, the diminishment of the original plant community species due to environmental stress may change the plant community structure and species composition. This was the case for *B. valdivianus*, which showed low tolerance the waterlogging condition occurred during winter (Stewart, 1996). In T1, after the waterlogging, *B. valdivianus* had a sharp decreased down to 50% of pasture DM, leaving empty spaces within the pasture, opportunity taken by unsown *L. perenne* and *T. repens* to growth and develop, increased up to 25%, and 15% of pasture DM contribution. The diminishment of *B. valdivianus* was reflected in the high SWC (low water uptake) at 40 cm soil depth for T1, between October and December of 2017.

In relation to *T. repens*, it did not have a great participation in any of the treatments in terms of botanical composition. For example, T2, was sown with *L. perenne* + *T. repens* and the maximum percentage of *T. repens* in botanical composition was around 18% during autumn 2016, in T1 (unsowed *T. repens*) reached same values during summer 2016, but in both treatments from that peak onwards it diminished constantly until it accounted by less than 10% during the winter 2016 (Figure 4.7). For this reason, *L. perenne* in T2 was considered as a monoculture across this thesis. Related to the presence of *T. repens* in T3, its participation was close to 15% of the pasture DM during summer 2019. In T4, *T. repens* was 30% of the botanical composition at the beginning of the study, but by winter 2017 it was less than 10%. In T5, *T. repens* across all seasons was insignificant as percentage of the pasture DM.

In T4, *B. valdivianus* made up to 40% of the botanical composition before winter, after the waterlogging it diminished down to 3%, leaving *L. perenne* to colonize the gaps, dominating the pasture during all the following seasons. However, during the soil water restriction, summer 2019, *B. valdivianus* increased its DM participation up to 30%, although the contribution of *B. valdivianus* was low after the waterlogging, however, was important enough to uptake more water (lower SWC) at 40 and 70 soil depth than T2. This shows the higher capacity of colonisation by *L. perenne*, described by Marriot et al. (1997) in comparison to *B. valdivianus*. The recovery of *B. valdivianus* reflected its high tolerance to water restriction periods (López et al., 2013; Ordóñez et al., 2018) and a proper regrowth due to the replenish of its water-soluble carbohydrates (Chapter 3).

In T5 pasture that was dominated by *L. perenne* and *P. lanceolata*, where *B. valdivianus* and *T. repens* had an exceptionally low participation in the pasture. In 5 of the 9 evaluated seasons were dominated by *P. lanceolata*; the other 4 seasons were co-dominated by *L. perenne* and *P. lanceolata*. When *L. perenne* population increased in T5 pasture, the water uptake at 10 cm soil depth also increased. These results were in line with those reported by Neal et al. (2012), which stated that *L. perenne* extracted less water at deeper soil horizons compared to *Bromus willdenowii*, *Phalaris aquatica* and *Lolium arundinacea*. Crush et al. (2005) indicated that *L. perenne* had a higher concentration of roots at 10 cm compared to *Dactylis glomerata*. This also explains the low water uptake by *L. perenne* at deep soil horizons in the present study. This co-dominance may have been due to the contrasting traits presented of both species, physiologically and roots traits differences. When *P. lanceolata* diminished its contribution to the pasture as a consequence of environmental constraints, i.e. low growth rate during low temperatures periods, autumn and winter (Teramura, 1978; Teramura et al., 1981), added to a low net photosynthesis (Sanderson et al., 2003), it was the increase of *L. perenne* presence, causing that T5 had

the highest water uptake at 10 cm of soil depth, this differences about growths rates are clearer when T2 (*L. perenne*) and T3 (*P. lanceolata*) are compared. However, when soil water restriction became a major environmental constraint, *P. lanceolata* increased in the pasture (Summer 2017; spring and summer, 2019), replacing the contribution of *L. perenne* and *T. repens* due to their depressed growth rate. It must be remarked that *P. lanceolata* dominated in both, T3 and T5, indicating a great capacity of persist, survive, and compete during the whole experimental period. This may be related to the grazing criterion used, where the grazing frequency was over 500 AGDD, the point where *B. valdivianus* replenished its WSC at leaf stage 4. Lee et al. (2015) indicated, that the replenish of WSC for *P. lanceolata* was at 532 AGDD, therefore the success of *P. lanceolata* during the experimental period, in T3 and T5, could be related to the WSC content at the moment of the grazing.

Lolium perenne dominated in T2 and T4, and co-dominate in T5. In T2 was expected to reach lower SWC values at 10 cm soil depth, as was showed by Ordóñez et al. (2018), situation that did not happen. Probably *L. perenne* phenotypic plasticity explains T2 SWC, which linked to the 3.5 leaf stage defoliation and the greater root intraspecific competition within *L. perenne* plants in T2, were the leading factors of a change in *L. perenne* root distribution, increasing root depth, being redistributed between the 10 and 30 cm soil depth.

The soil water dynamics, from the surface up to 70 cm soil depth, directly reflected the botanical composition of the pasture. Functional species traits explain SWC variation at 40 and 70 cm soil depth (i.e. root traits) (Neal, 2012; Ordóñez et al., 2018) and probably differences in physiological traits (i.e. stomatal conductance) (Blum, 2009). A diverse pasture provides a wide range of root traits, hence a more effective soil water extraction

than a pasture based on *L. perenne* and *T. repens* (Ordóñez et al. 2018). The pastures with higher deep-rooted species abundance were more effective using soil water across the seasons.

Across the soil water restriction periods, fluctuations in soil water content were assessed, as an indication of a possible redistribution of water in the soil profile. Water lifting results in water movement from the roots to the soil, due to a large difference in soil matric potential between the root and the soil which may occur when the soil surface has a high matric potential (Richards and Caldwell, 1987; Caldwell and Richards, 1989; Caldwell et al., 1991; Caldwell et al., 1998; Skinner, 2004; Skinner et al., 2009). In the present study, when the soil reached less than 16% of SWC (around 2000 kPa of soil matric potential) at 10 cm soil depth, a variation in SWC occurred, between the 08:00 and 17:00 hours, reached a maximum difference of 0.58% of SWC. However, the quantification of the water lifted is complex, because it is the outcome of several processes involved, in this case, evaporation and transpiration (Simunek et al., 2003). Therefore, it is possible to assume that the quantity of water lifted by the plants is higher than that observed in the present study. Further research needs to be undertaken on this subject to determine if the input of water generated by the “water lifting” has any ecological and productive outcomes.

4.5 Conclusions

Bromus valdivianus and *P. lanceolata* have a higher tolerance to soil water restriction than *L. perenne*. Both species have structural and physiological mechanisms that allow them to generate a higher herbage mass production during the soil water restriction and are more effective extracting water from deeper soil layers than *L. perenne*. The photosynthesis rate, leaf osmotic and leaf water potentials were indications that *B.*

valdivianus and *P. lanceolata* were under less stress during soil water restriction periods than *L. perenne*.

Bromus valdivianus low herbage mass contribution in T4 was enough to increase the soil water uptake at 40 and 70 cm soil depth in comparison to *L. perenne* grown as monoculture (T2). *Bromus valdivianus* showed a low capacity to compete against *L. perenne* and *P. lanceolata*.

The soil water dynamics was reflection of the species root traits and its contribution within each treatment. Differences in SWC were more clearer during soil water restriction periods and when soil depth was greater.

Pasture species that differed in their ecosystem function, conferred a higher biomass production during the seasons and across water restriction periods (e.g. T5 growth rate compared to T2 during summer; T5 growth rate compared to T3 during autumn and spring), which was shown by changes in the botanical composition of *P. lanceolata* and *L. perenne* throughout the study.

Signs of “water lifting” were recorded during the soil water restriction periods, which was consistent across the three consecutive soil water restrictions periods measured. Further research on this topic needs to be undertaken, to understand its importance in plant survival and growth, and in plant communities with higher functional diversity.

Chapter 5 Experimental chapter

Chapter 5 Tiller population dynamics and photosynthetic carbon fixation capacity for *Lolium perenne* L. and *Bromus valdivianus* Phil. under monoculture and intra specific competition in field conditions.

Summary

- The partition of the resources in grasses is directed to leaf, root and tiller appearance and growth. In *L. perenne* is suggested that tillering has a low priority, therefore any tiller population changes reflects the interaction between the environmental conditions and the defoliation management due to grazing. The present study determined the tiller population dynamics (vegetative reproduction), competition ability and potential carbon fixation capacity for *B. valdivianus* and *L. perenne*.
- The treatments were : T1) *B. valdivianus*; T2) *L. perenne* + *T. repens* and T3) *B. valdivianus* + *L. perenne* + *T. repens*. Grazing events were performed when *B. valdivianus* reaches a leaf stage 4. Tiller populations of *B. valdivianus* and *L. perenne* in all treatments were measured within fixed quadrat positions, besides leaf area and tiller weight evaluated. Leaf area index and photosynthetic carbon fixation capacity (PCFC) were estimated.
- *Bromus valdivianus* tiller population diminished during the winter, however by the end of the summer period *B. valdivianus* had recovered in T1 and T3. *Lolium perenne* was sensitive to soil water restriction having a high tiller mortality during the summer period. During all critical periods, was estimated that *B. valdivianus* + *L. perenne* mixed pasture (T3) generally reached higher values of PCFC than T1 and T2.
- *Bromus valdivianus* tiller population diminished during the soil water restriction post waterlogging, but was tolerant to soil water restriction. *Lolium perenne* tolerated winter waterlogging but not the summer soil water restriction period. When the soil water restriction stress was released, *L. perenne* experimented a fast recovering. Both species showed asynchrony in their tiller population growth due to their different critical periods. T3 showed a higher soil water uptake than T2 but less than T1.

5.1 Introduction

Plant stress has been defined as an excess or deficit of solar radiation, water, nutrients or an excess of toxins in the environment that negatively affects plant physiology and development (Grime, 1977). A plant community can be formed by many individual plants of different species, as is the case of a pastures, where competition is a process that alters and determines plant community structure and ecological dynamics (Hortal et al., 2017). The competition outcome is a function of the stress intensity, as mediated by nutrient and water availability, and the environmental disturbance, independently of their origin (Tilman, 1988; Pugnaire et al., 2004). The effects of stress on plant traits negatively affects the survival and persistence of plants. A more diverse pasture increases the probability that stress-tolerant traits are present, increasing the resistance to environmental constraints (Petchey et al., 2015; DeClerck et al., 2016). Therefore, different plants traits drive different ecological processes (Tilman et al., 1999; Isbell et al., 2009), and through their action they may alter the ecosystem, for example, affecting the soil water conductivity (Moreno-Espindola et al., 2007) or soil water dynamics (Gan et al., 2012; Ordóñez et al., 2018).

A deep root system is a key trait for increasing plant species tolerance and survival to water stress periods (Blum, 2009), therefore, species with deeper root distribution, such as *B. valdivianus* in comparison to *L. perenne* (Lopez et al., 2013; Ordóñez et al., 2018; Descalzi et al., 2019; Chapter 4) should have greater survival during water restriction periods. A functionally diverse pasture, in terms of root traits, creates a more even water uptake through the soil profile, due to a more thorough soil exploration due to deeper roots (Ordóñez et al., 2018; Descalzi et al., 2019). Therefore, in a temperate humid climate, which normally has a water deficit period in summer, perennial pasture species

with deep root distribution have a greater chance of surviving the deficit. On the other hand, shallow-rooted species in a pasture, such as *L. perenne*, help to increase nitrogen uptake (Crush et al., 2005; Crush et al., 2009) and if a species is well adapted to waterlogging (Etherington, 1984; McFarlane et al., 2003; Jurczyk et al., 2016) it will help to maintain high levels of production in climates with well-marked rainfall seasons. Mixing the species according to the different root traits, such as shallow and deep root traits, could be a valid strategy to increase the functional diversity of the pasture and increase pasture stability at a low plant diversity (Hooper and Vitousek, 1997; Chapter 4) under different water stress periods (waterlogging and water restriction).

Bromus valdivianus naturally grows in free-draining volcanic soils (Andisols) with high fertility levels, being able to compete with *L. perenne* in pastoral systems in the south of Chile (López et al., 1997). In relation to *L. perenne*, *B. valdivianus* has greater water uptake at both 40 cm and 70 cm soil depth, lower leaf osmotic potential, lower leaf water potential, similar annual accumulated herbage mass production, similar photosynthesis rate under field conditions (Chapter 4), similar forage quality (Balocchi and López, 1996), higher leaf area and tiller weight (López et al., 2013; Ordóñez et al., 2017; Chapter 3) and a higher number of live leaves per tiller at any one time, but with fewer tillers per area (Ordóñez et al., 2017; Chapter 3). *Bromus valdivianus* expressed its low tolerance to waterlogging (Stewart, 1996; Chapter 4), on the other hand, it had a greater growth under dry conditions than *L. perenne* (López et al., 2013; Ordóñez et al., 2018; Chapter 4). In contrast, *L. perenne* grew better under waterlogging and low temperatures than *B. valdivianus* (Chapter 4). These insights indicate that *B. valdivianus* is a species suitable for dairy, beef and sheep production systems, with the advantage that it can increase production and confer greater pasture stability during drought periods (Keim et al., 2014).

Individual tiller appearance and tiller density are an essential part of the pasture growth (Milthorpe and Davidson, 1966). Therefore, tiller population dynamics over time are an indication of the environmental conditions-pasture species relationship (Hernández-Garay et al., 1997b), especially because changes in tiller population can be rapid in response to changing environmental conditions (Bullock et al., 1994).

Commonly, pasture grasses have been grazed according to *L. perenne* defoliation criteria, which are based on leaf stages, between 2.5 and 3 leaf stage (Fulkerson and Slack, 1994a,b; Donaghy and Fulkerson, 1997,1998; Turner et al., 2006a), or on time frequency (Tainton, 1974; Motazedian and Sharrow, 1987) as was indicated by Fulkerson and Slack (1994a,b), with the common output being the encouragement of *L. perenne* persistence (Donaghy and Fulkerson, 1997,1998). Either way leaf stage and herbage mass production have been related (Chapman et al., 2011), and farmers defoliate pastures according to herbage mass criteria or the leaf stage criterion recommended by Donaghy and Fulkerson (1997,1998). However, the current managements and criteria for pasture do not consider the plant total carbon fixation (root plus shoot; Wang et al., 2007; Pausch and Kuzyakov, 2018), which should be considered by both researchers and farmers, especially when an important objective by the COP21 and UN (2030 agenda for sustainable development) is to look for sustainable ways for food production.

Two species were selected according to their contrasting root traits: *L. perenne* as a species with shallow root distribution and *B. valdivianus* as a species with deep root distribution (Chapter 4). The aim of this study was to determine the above-ground growth and total carbon fixation of *B. valdivianus* and *L. perenne* in relation to fluctuations in annual environmental conditions under monoculture and in mixed swards. To accomplish

this objective, tiller population dynamics, tiller leaf area, accumulated herbage mass, botanical composition, soil water content and photosynthesis were measured.

5.2 Materials and methods

5.2.1 Geographic location and weather description

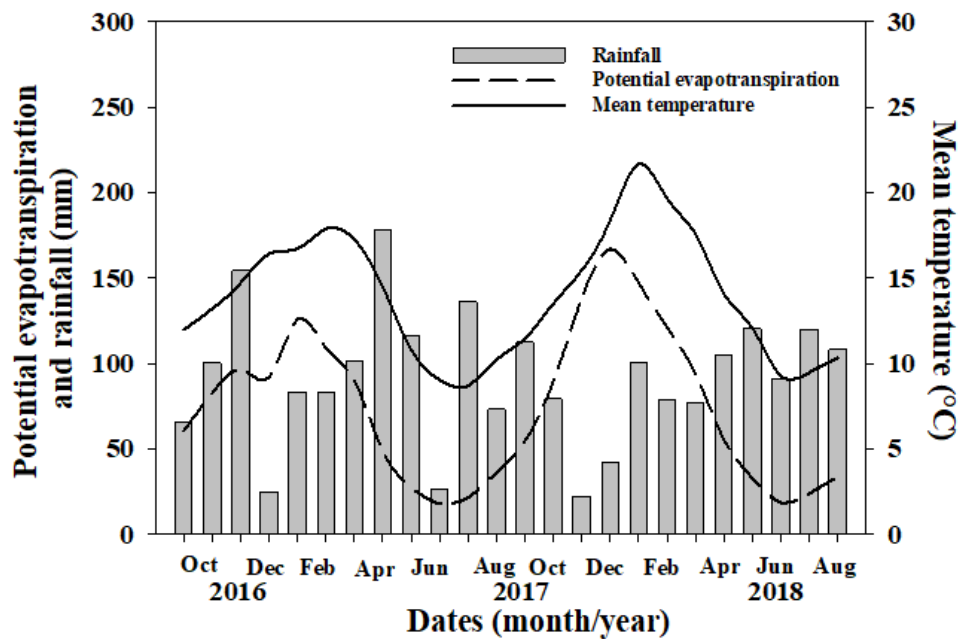


Figure 5.1 Rainfall, mean monthly air temperature and mean monthly potential evapotranspiration estimated with the Penman Monteith equation during the experimental period, between September 2016 and August 2018.

The study was performed at Massey University Dairy One Farm, Palmerston North (40°22'55.8"S 175°36'23.8"E), in a Manawatu fine sandy loam soil (Dystric Fluventric Eutrochrept), a Weathered Fluvial Recent Soil (Hewitt, 1998). Plots were sown on the 20th October 2016, and the experimental period was performed between 01/03/2017 and 19/07/2018. Weather data were collected from a weather station located 200 meters from the experimental site (Agent number 21963; authorship to AgResearch), where soil water content (SWC) at 20 cm depth, soil temperature, radiation are shown in Figure 5B. Air

temperature, rainfall and evapotranspiration were recorded during the experimental period (September 2016 to August 2018) are shown in Figure 5.1.

5.2.2 Treatments description

The experiment consisted of three types of pastures, T1) *B. valdivianus* (cv. Bareno; sown at 30 kg ha⁻¹); T2) *L. perenne* (cv. Rohan; sown at 25 kg ha⁻¹) + *Trifolium repens* (cv. Weka; sown at 3 kg ha⁻¹); T3) *B. valdivianus* (sown at 20 kg ha⁻¹) + *L. perenne* (sown at 12 kg ha⁻¹) + *T. repens* (sown at 3 kg ha⁻¹), established in 110 m² plots according to a randomised complete block design (4 blocks x 3 treatments). The soil bed preparation consisted of soil ploughing and harrowing after which the pastures were sown using a direct seed drill. Defoliation was undertaken with 5 sheep grazing per plot, equivalent to a stocking density of 454 sheep ha⁻¹. The grazing interval was at leaf stage 4 of *B. valdivianus* plants (Chapter 3; Ordóñez et al., 2017).

5.2.3 Botanical composition and herbage mass

Herbage mass was measured before and after grazing according to the methodology described by Earle and McGowan (1979). Eight herbage samples of 0.1 m² quadrat per treatment were collected (2 samples per plot) by cutting the pasture down to ground level before and after grazing. The samples were oven dried at 70°C for two days and then weighed to express yield on a dry matter (DM) basis. Chapter 4 describes further details regarding to herbage mass measurements and determination.

Botanical composition was measured using the method described by Radcliffe et al. (1968). For each botanical composition event, two pasture samples of 0.1 m² quadrat per plot were cut down to at ground level once a season, from summer 2017 until summer 2019 (The second month of each season). The species (*B. valdivianus*, *L. perenne*, *T.*

repens, other grasses, broad leaf species) were manually separated, placed into individual bags, oven dried at 70°C for two days or until reaching constant weight, and then weighed. The contribution of *B. valdivianus* (in T1 and T3) and *L. perenne* (in T2 and T3) for the pasture accumulated herbage mass was determined, allowing to evaluate the competition index (see section 5.2.5 and equation 5.2) for both species in terms of herbage dry mass. Chapter 4 describes further details regarding botanical composition measurements.

5.2.4 Tiller population dynamics

All the measurements related to tiller population, leaf area and photosynthesis were done for *B. valdivianus* monoculture (T1) and mixture (T3) and *L. perenne* in monoculture (T2) and mixture (T3) in the 4 blocks. Three metallic rings (10 cm diameter, equating to 78.5 cm²) were placed in fixed positions per plot, according to each treatment, visually targeting a species cover of 100% of *B. valdivianus* (T1), 100% *L. perenne* (T1) and 50% of each species in the mixture (T3), leaving off weeds or any other species. The alive tiller number contained within each ring was counted every two weeks. The rings were considered subsamples within each plot.

Each ring had three metallic 1.5 cm legs welded underneath, and a 150 x 6.0 mm flat head galvanised nail was inserted in the soil to indicate each ring leg position. A cleft was made to each nail head, so that each ring leg perfectly fitted. After finishing recording a ring tiller population, the ring was removed, and its position remained permanently marked by the three nails inserted in the ground. At any measuring event, the nails were found by using a metal detector (F22 Metal Detector, Fisher Research Labs, USA) and the ring was placed in position, so its legs matched the three nail head clefts. Therefore, at every measuring event the same tiller population was evaluated, allowing the tiller population changes over time to be captured. This method provided a minimum degree of disturbance

to the sampling location, and tiller counting occurred every 2 weeks. The evaluation were independent of the grazing events. Grazing events are shown in the figure 4.2.

The results of the competition intensity between *L. perenne* and *B. valdivianus* were obtained by contrasting *B. valdivianus* and *L. perenne* in T3 with T1 and T2, respectively, over time. The collected data were treated following the methodology of Bullock et al. (1994) and Flores et al. (2017), which meant that the population recorded in the first evaluation was considered as 100% and all the population density changes over time were expressed as a percentage in relation to the initial population.

5.2.5 Competition index

Competition intensity was determined by using the relative competition indices, which are shown in equation 5.1 and 5.2 (RCI; Wilson and Tilman, 1993; Grace, 1995). Equation 5.1 describes the RCI related to tiller populations and equation 5.2 describes the RCI related to herbage mass accumulation. The two equations provided complementary approaches to explain the fluctuations in tiller population and herbage mass (Chapter 4) through the evaluation period due to competition. The following equations described these relationships:

$$\mathbf{RCI_T = (P_{TM} - P_{TC})/P_{TM} \quad (eq. 5.1)}$$

$$\mathbf{RCI_H = (P_{HM} - P_{HC})/P_{HM} \quad (eq. 5.2)}$$

Where:

RCI_T = relative competition index for tiller population per season.

P_{TM} = tiller population under the monoculture treatments for *B. valdivianus* (T1) or *L. perenne* (T2).

P_{TC} = tiller population for *B. valdivianus* or *L. perenne* in the mixture (T3).

RCI_H = relative competition index for accumulated herbage mass per season.

P_{HM} = accumulated herbage mass under the monoculture treatments for *B. valdivianus* (T1) or *L. perenne* (T2).

P_{HC} = total accumulated herbage mass for *B. valdivianus* or *L. perenne* in the mixture (T3).

A relative competition index (RCI) of zero means that the monoculture and mixture provided the same number of tillers, or herbage mass, while a negative RCI means that one of the species had a higher contribution in the mixture (T3) than in the monoculture, T1 or T2, indicating that intraspecific competition is greater than the interspecific competition.

5.2.6 Tiller weight and tiller leaf area

To determine tiller structural differences between *B. valdivianus* and *L. perenne*, five tillers were harvested, in five different periods in each plot, 30/03/2017; 14/04/2017; 30/04/2017; 20/07/2017; 28/07/2017. The tillers harvested were of similar development, with no signs of stress and avoiding the daughter tillers of each species. Tiller leaf area, tiller weight and leaf stage development were determined.

Due to the sampling of tillers using a fixed location the real tiller population per m² may have been overestimated (Hernández-Garay et al., 1997b; Bullock et al., 1994; Flores et al., 2017). During August 2019, 4 pasture cores of 100 cm² with 15 cm of depth were randomly collected in each plot in order to determine in laboratory the tiller population inside each core, leaf stage and leaf area for both species within the core (Mitchell and Glenday, 1958), afterwards all plant material was oven dried at 70°C for 48 hr and the

tiller dry matter was obtained. The aim of this was to relate the tiller population within the core against the tiller population within the fixed positions. The leaf area of the individual tillers along with the leaf area within the cores, were determined using a LI-3100C area meter (LI-COR, Nebraska, USA) for both species.

5.2.7 Soil water content dynamics

To determine the relationship between soil water content (SWC) and tiller population dynamic of the species evaluated, SWC sensors (Campbell scientific; model 616) were installed at 10 and 40 cm soil depth and monitored with a datalogger (Campbell scientific CR1000) together with a multiplexer (Campbell scientific; model 16/32B). Thus, two sensors of each type were placed at each soil depth. The average SWC was determined between every tiller population evaluation. The SWC and tiller population dynamics relationships were explored between 11/10/2017 and 19/07/2018.

5.2.8 Photosynthetic carbon fixation capacity

Photosynthetic carbon fixation capacity (PCFC) was estimated using the equation developed by Zheng et al. (2011) as follows:

$$\text{PCFC} = (A_n * \text{LAI} * 12 * 10,000 * 3,600) * 10^{-6}/1,000 \quad (\text{eq. 5.3})$$

Where:

PCFC = photosynthetic carbon fixation capacity

A_n = is the net photosynthesis ($\mu\text{mol m}^2 \text{s}^{-1}$) determined during different seasons under different weather conditions (Chapter 4).

LAI = leaf area index, estimated with the total tiller population per m^2 and leaf area of every leaf stage (equation 4; Table 2).

10,000 = mathematical constant that transforms collected information from m² to ha.

3,600 = mathematical constant that transform information from seconds to hour.

10⁻⁶ = mathematical constant to transform from μmol to mol.

12 = atomic mass of C used to transform data from mol to grams.

1,000 = allowed to transform from grams to kilograms.

5.2.9 Tiller population correction and estimated leaf area

When tiller population is evaluated using a fixed location (as in this study), tiller density may be overestimated, because the empty spaces between plants and between sown lines are not included in the fixed location (Bullock et al., 1994; Hernández-Garay et al., 1997b; Descalzi, 2011; Ordóñez, 2013). As one of the objectives was to determine the tiller population dynamics due to both changing environmental conditions and intra and interspecific competition, it makes sense not to include the empty pasture spaces. The problem is an overestimation of tiller population when the data are translated to m². Therefore, the steps to estimate the changing leaf area index (LAI) between every tiller population measurement were as follows: 1) A tiller population correction factor was determined for *B. valdivianus* and *L. perenne* comparing the tiller population within the fixed quadrats and the tiller population within the core (eq. 5.5; Table 5.1); 2) tiller leaf area and leaf stage inside the core were determined (Table 5.1) and compared. With the information of leaf development presented in the Figure 5.2, with the leaf development (leaf stage) as a function of accumulated growing degree days (AGDD) it was possible to; 1) Determine the leaf stage development in function of AGDD for both species (Figure 5.2); 2) Using the information within the Table 5.1, where the leaf area is related to the leaf stage, a relationship between the leaf area development and AGDD was

developed, resulting in the equations showed in Table 5.2, where the leaf area development was estimated with the accumulated growing degree days determined with temperature data from the climatic station (Agent number 21963; authorship to AgResearch).

Table 5.1 Tiller number, leaf area per tiller in each core of 100 cm² taken during August 2019.

Species	Leaf stage	Tiller number (100 cm ²)	SEM Tiller number (100 cm ²)	Tiller leaf area (cm ² tiller ⁻¹)	SEM Tiller leaf area (cm ² tiller ⁻¹)
<i>B. valdivianus</i>	2.0	56.7	48.2	6.3	3.4
	2.5	35.6	7.7	5.3	1.5
	2.75	40.8	20.4	10.7	3.8
	3.0	43.5	15.7	8.1	1.9
	3.5	29.0	14.5	13.0	6.5
<i>L. perenne</i>	2.0	75.3	30.6	3.1	0.3
	2.5	54.9	13.2	3.9	0.8
	2.75	60.6	9.6	3.7	0.5
	3.0	75.5	11.0	3.9	0.3

The LAI for both species was estimated according to the following equation:

$$\text{LAI} = \text{TP}_c * \text{TLA} \quad (\text{eq. 5.4})$$

Where:

LAI = leaf area index.

TP_c = corrected tiller population per m².

TLA = tiller leaf area, according to the different leaf stage development given by AGDD.

Equations are shown in Table 5.2.

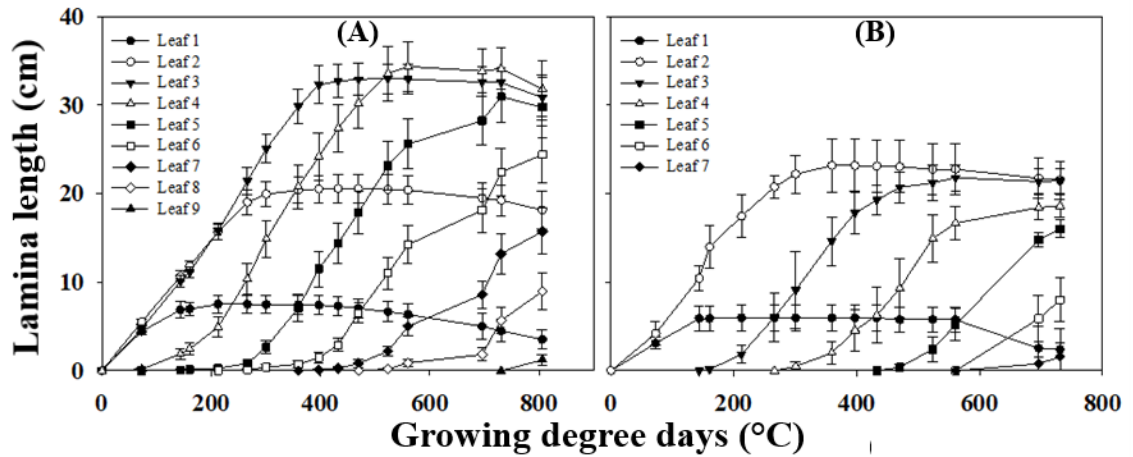


Figure 5.2 Tiller leaf growth dynamics and lamina length for *B. valdivianus* (A; Chapter 3) and *L. perenne* (B). Bars indicate standard error of the mean (n=6). Base temperature 0° C.

Tiller population correction factor (TPCF) was estimated according to the following equation:

$$\text{TPCF} = \text{TP}_{\text{fl}} / \text{TP}_{\text{core}} \quad (\text{eq. 5.5})$$

Where:

TPCF = tiller population correction factor for the tiller population.

TP_{fl} = tiller population within the fixed locations per m² from the winter evaluations.

TP_{core} = tiller population within the cores per m², the samples collected in August.

Tiller population correction (TPCF) was used for every tiller population evaluation, keeping the proportions of the original data of the fixed locations but with a realistic tiller population per m².

The corrected tiller population (TP_c) was estimated according to the following equation:

$$\mathbf{TP_c = TP_{fl} / TPCF \quad (eq. 5.6)}$$

Where:

$\mathbf{TP_c}$ = corrected tiller population for every measurement translated to m^2 .

$\mathbf{TP_{fl}}$ = original tiller population inside the fixed location (or inside the ring) per m^2 .

\mathbf{TPCF} = tiller population correction factor estimated in the equation 5.

Using the equation 5.5, where the tiller population from August from the fixed quadrats ($\mathbf{TP_{fl}}$) and tiller population from the cores ($\mathbf{TP_{cores}}$) resulted in factor of 3, in other words, the fixed quadrats overestimate the tiller population per m^2 by 3. According to the literature, *L. perenne* population were between 4 and 8 thousand tillers per m^2 , depending the season (Hernández-Garay et al., 1997a,b; Matthew et al., 2000; Hendriks et al., 2016).

5.2.10 Relationship between the leaf stage development and leaf area index

Using the equations presented in Table 5.2 and the tiller population corrected, the LAI was estimated. The strength of this estimation made it possible to estimate the changes in LAI according to tiller population dynamics and the tiller leaf development after every grazing. Therefore, LAI is equal to tiller population per tiller leaf area development. The LAI estimated in this research was similar to the results reached by King et al. (1984), suggesting a reliable estimation of this variables (Figure 5.7B).

5.2.11 Net photosynthesis values for the estimation of photosynthetic carbon fixation capacity

The net photosynthesis values used for the PCFC estimation were taken from the Chapter 4 and an average per season was calculated. The values were; summer (water restriction): $7.9 \mu\text{mol m}^2 \text{s}^{-1}$ for *B. valdivianus* and $5.6 \mu\text{mol m}^2 \text{s}^{-1}$ for *L. perenne*; autumn: $23.3 \mu\text{mol m}^2 \text{s}^{-1}$

$\text{m}^2 \text{s}^{-1}$ for *B. valdivianus* and $28.4 \mu\text{mol m}^2 \text{s}^{-1}$ for *L. perenne*; winter: $15.5 \mu\text{mol m}^2 \text{s}^{-1}$ for *B. valdivianus* and $15.7 \mu\text{mol m}^2 \text{s}^{-1}$ for *L. perenne*; early spring: $23.3 \mu\text{mol m}^2 \text{s}^{-1}$ for *B. valdivianus* and $28.4 \mu\text{mol m}^2 \text{s}^{-1}$ for *L. perenne*; late spring: $11.2 \mu\text{mol m}^2 \text{s}^{-1}$ for *B. valdivianus* and $7.12 \mu\text{mol m}^2 \text{s}^{-1}$. This variable were evaluated between 11 am - 2 pm, to evaluate the species during the higher level of atmospheric water demand, reflecting differences between species due to the different effectiveness of soil water uptake (Blum, 2009). The gas exchange was evaluated using a LICOR-6400XT (LI-COR, Nebraska, USA), chamber 6400-02B LED light source (LI-COR, Nebraska, USA). The photosynthetic photon flux density at the leaf surface was set at $1,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, leaf temperature was stabilised to 25°C , CO_2 flow rate of $500 \mu\text{mol s}^{-1}$ and the CO_2 concentration ambient of incoming gas stream set at $400 \mu\text{mol s}^{-1}$. The days chosen for the field measurements had no rainfall or risk of rainfall forecast. Therefore, PCFC is equal to LAI per net photosynthesis (A_n) of *B. valdivianus* and *L. perenne* across every evaluation done.

Table 5.2 Equations relating the tiller leaf area (Table 5.1) with the accumulated growing degree days (Figure 5.2A and 5.2B) for *B. valdivianus* and *L. perenne*. The “x” is the accumulated growing degree day (base temperature 0°C) and “y” is the tiller leaf area at a given accumulated growing degree day.

species	Equations	r^2
<i>B. valdivianus</i>	$y = 0.00002x^2 + 0,0129x + 0,0313$	0.88
<i>L. perenne</i>	$y = -0,000009x^2 + 0,0119x - 0,0072$	0.99

Note: The maximum “x” value for the equation is 445 growing degree days, the time for *B. valdivianus* to reach leaf stage 4 and for *L. perenne* reach leaf stage 3.5 under field conditions using base temperature of 0°C (McMaster and Wilhelm, 1997). These are the higher leaf stages for grazing in each species.

5.2.12 Experimental design and statistical analysis

The study was designed according to a randomised complete block design (3 treatments x 4 blocks) and the blocks were set up according to the slope of the experimental site. Normality of the data was determined by using the Shapiro Wilk's test and the homogeneity of variance. The ANOVA analysis was performed on normally distributed data. Fisher's LSD test was applied to separate the treatment means. For Non-normal data, the median was compared, with Kruskal-Wallis analysis (total tiller population). Pearson correlation was used to analyse the association between tillering and climatic variables.

Tiller population, LAI and PCFC were compared using the standard error of the mean (\pm sem). The initial tiller population was considered as 100%, thus comparing each individual measuring event with the initial population captured the successive fluctuations through time.

5.3 Results

5.3.1 Relative changes in tiller population dynamics related to soil water content dynamics

The relative tiller population changes (starting from an assumed baseline of 100%) are shown in Figure 5.3. In late winter 2017 (August), the *B. valdivianus* monoculture (T1) tiller population peak was 146% (± 12 ; Figure 5.3A), while the tiller population of *B. valdivianus* in the mixture (T3) increased up to 221% (± 25). During winter, the soil reached saturation, with (mean \pm sem) 43.5% SWC (Figure 5.3B). Once soil saturation was reached, it fluctuated between 43.5% and 41.7% SWC until October 2017 (Figure 5.3B; Figure 5.3C; Figure 5.3D). During the saturation period, *B. valdivianus* relative

tiller population started to decrease, starting in October 2017 until December 2017 reaching the lowest values of 47% (± 18) in T1 and 49% (± 28) in T3, matching with the lower SWC (14.6%) on December (18/12/2017; Figure 5.3B). Between December and March, *B. valdivianus* tiller population recovered reaching 106% (± 13) for T1 and 88% (± 36) for T3.

Table 5.3 Pearson correlation coefficient for soil water content (SWC), air temperature (Air °C), soil temperature (Soil °C) and rainfall related to tiller number for *B. valdivianus* in T1 and T3, *L. perenne* in T1 and T3.

	Species			
	<i>B. valdivianus</i> (T1)	<i>B. valdivianus</i> (T3)	<i>L. perenne</i> (T2)	<i>L. perenne</i> (T3)
SWC	0.715	0.814	0.632	-0.305
p-value	<0.0001	<0.0001	<0.0001	0.0894
Air °C	-0.484	-0.669	-0.535	0.227
p-value	0.0049	<0.0001	0.0016	0.210
Soil °C	-0.554	-0.690	-0.534	0.219
p-value	0.0013	<0.0001	0.0017	0.227
Rainfall	0.237	0.066	0.135	0.104
p-value	0.1907	0.719	0.461	0.567

Lolium perenne in the monoculture (T2) reached a peak in October 2017 of (mean \pm sem) 214% (± 24.4) and for *L. perenne* in the mixture (T3) a peak in November 2011 of 240% (± 49). The large decrease in SWC, reaching 14.6% SWC (Figure 5.3B and 5.3C) in December 2017 (18/12/2017; Figure 5.3B), matched the greatest decrease in *L. perenne* tiller populations, 100% (± 28) in T2 and 188% (± 28) in T3. However, after rain at the end of December 2017, *L. perenne* tiller populations increased sharply; *L. perenne* in T3 reached a peak of 343% (± 12) in January 2018 (26/01/2018). Although, immediately after reaching the peak, the relative tiller population of *L. perenne* had a sharply decrease, matching with the diminishment of SWC (Figure 5.3).

During March 2018 an increase in SWC (over 30%) and lower atmospheric demands (Figure 5.2), *L. perenne* tiller populations increased reaching, at the end of the evaluation on the 19/07/2018, 148.3% (± 9.5) in T1 and 240.8% (± 52.6) in T3.

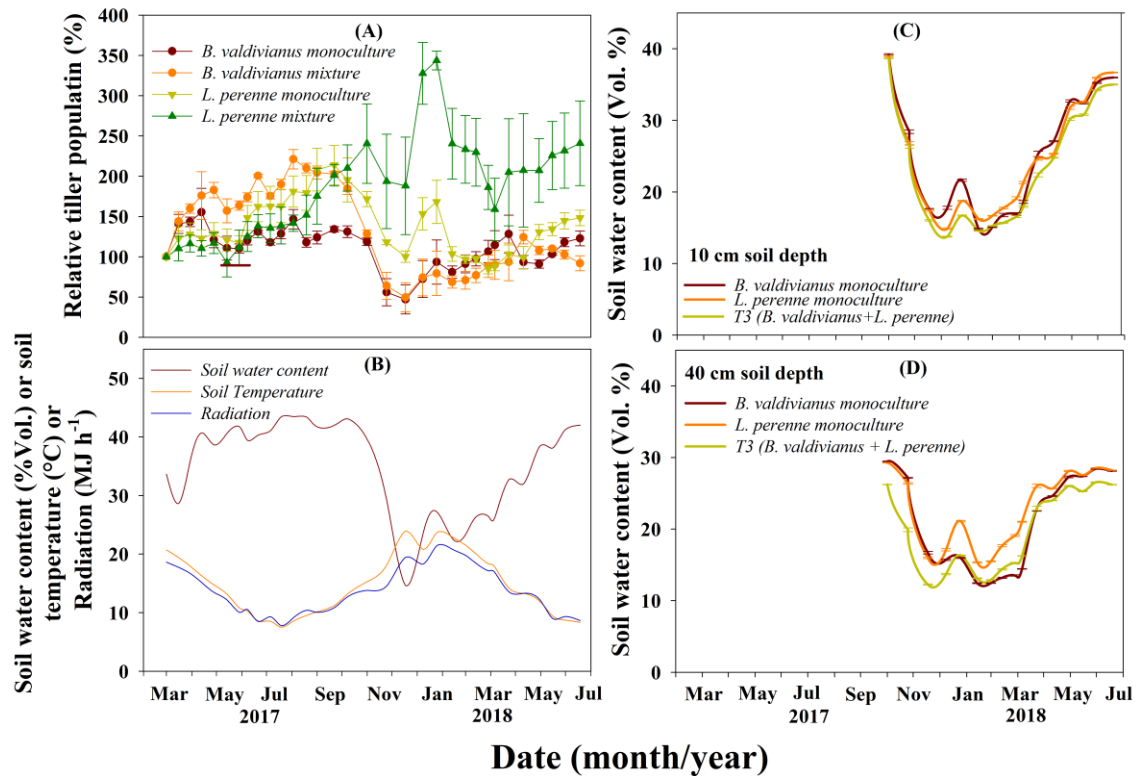


Figure 5.3 Relative tiller population dynamics for *B. valdivianus* in T1 and T3 treatments and *L. perenne* in T2 and T3 treatments (A). Soil temperature, soil water content at 20 cm (SWC) and radiation (B). Soil water content (SWC) at 10 cm for T1 (*B. valdivianus*), T2 (*L. perenne*) and T3 (*B. valdivianus* + *L. perenne* mixture) (C). Soil water content (SWC) at 40 cm for T1 (*B. valdivianus*), T2 (*L. perenne*) and T3 (*B. valdivianus* + *L. perenne* mixture) (D). Bars indicate standard error of the mean, for A (n=3) and for B (n=96).

Significant differences between were assessed (Figure 5.3A). Relative tiller population was higher ($p \leq 0.05$) for *B. valdivianus* in T3 compared to T1 until November 2017. Subsequently relative tiller population for *B. valdivianus* in T1 and T3 were similar

($p > 0.05$). During the first winter until the beginning of October (during the soil water saturation), the relative tiller populations for *L. perenne* were statistically similar ($p > 0.05$). However, there was a significant increase compared to *L. perenne* in T2, from November 2017 until the end of the evaluation 19/07/2018.

Soil water content (SWC) is an indication of soil water uptake and are inversely proportional. Figure 5.3D shows that *B. valdivianus* (T1) and the *B. valdivianus* + *L. perenne* mixture (T3) extracted more water at 40 cm soil depth compared to *L. perenne* (T2), especially when SWC reached less than 20%, between December 2017 and April 2018, with two sharp SWC decreases, one in December and the other during February. In December *B. valdivianus* + *L. perenne* mixture (T3) had the lowest values, followed by *L. perenne* (T2) and *B. valdivianus* (T1). Between February and April 2018 *B. valdivianus* (T1) had the lowest values, followed by *B. valdivianus* + *L. perenne* (T3) and thereafter *L. perenne* (T2), indicating a higher water uptake by T1. This may indicate full recovery in *B. valdivianus* (T1) functional roots, it is assumed because it matches with tiller populations recovery.

For *B. valdivianus* (T1) the highest values of SWC at 40 cm soil depth were recorded between October 2017 and the beginning of December 2017. Matches with its high death rates and a sharp population diminish. On the other hand, between mid-December 2017 and April 2018, *B. valdivianus* (T1) tiller populations started to increase, as well as the SWC at 40 cm depth reached the lowest values (higher water uptake). From April and onwards *B. valdivianus* + *L. perenne* (T3) had the lowest SWC.

5.3.2 Tiller population variation and its relation to environmental factors

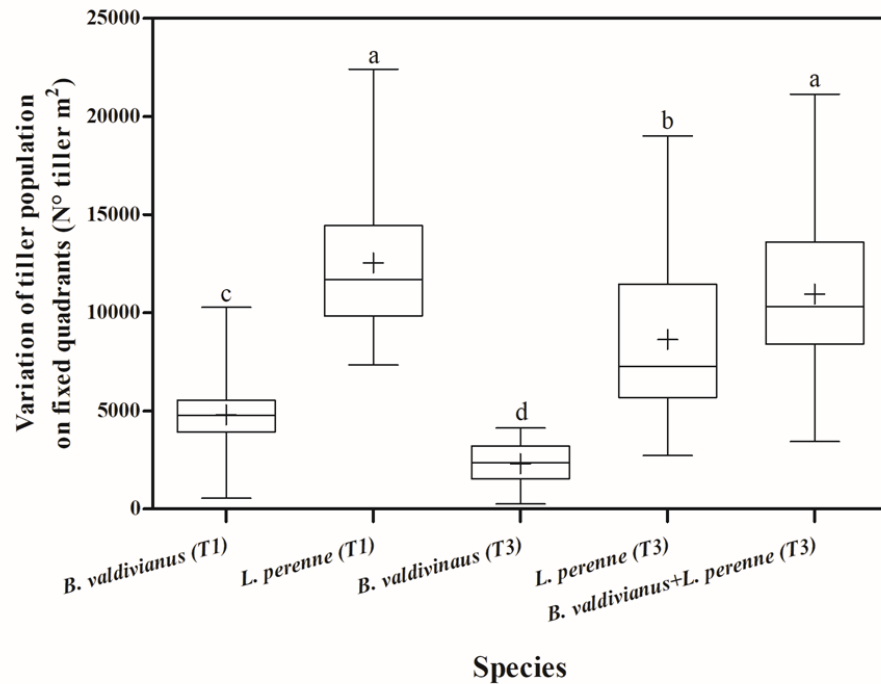


Figure 5.4 Tiller total population for *B. valdivianus* in T1 and T3, *L. perenne* in T2 and T3 and the total tiller population of T3 (sum of *B. valdivianus* in T3 and *L. perenne* in T3). Lowercase letters indicate statistical differences. Boxes and bars indicate quartiles and median, + indicate the mean of the population (n=288).

The variation in the total tiller population per m², in the fixed positions, for the whole evaluation, 01/03/2017 and 19/07/2018 is shown in Figure 5.4. Differences were significant, between species ($p \leq 0.001$) and when *B. valdivianus* ($p \leq 0.05$) monoculture and the mixture were compared. *Bromus valdivianus* had lower tiller numbers than *L. perenne* with a median of 4791 vs. 12545 tillers m⁻² in monoculture and 2309 vs. 8638 tillers m⁻² in the mixture ($p \leq 0.01$), respectively. The coefficient of variation for monocultures was lower in comparison to the mixtures, in such a way that for the *B. valdivianus* monoculture was 28.8% and for *L. perenne* was 30.5 %, compared to *B.*

valdivianus in the mixture at 45.1% and *L. perenne* 44.1%. The coefficient of variation for the *B. valdivianus* + *L. perenne* mixture (T3) was 34.0%.

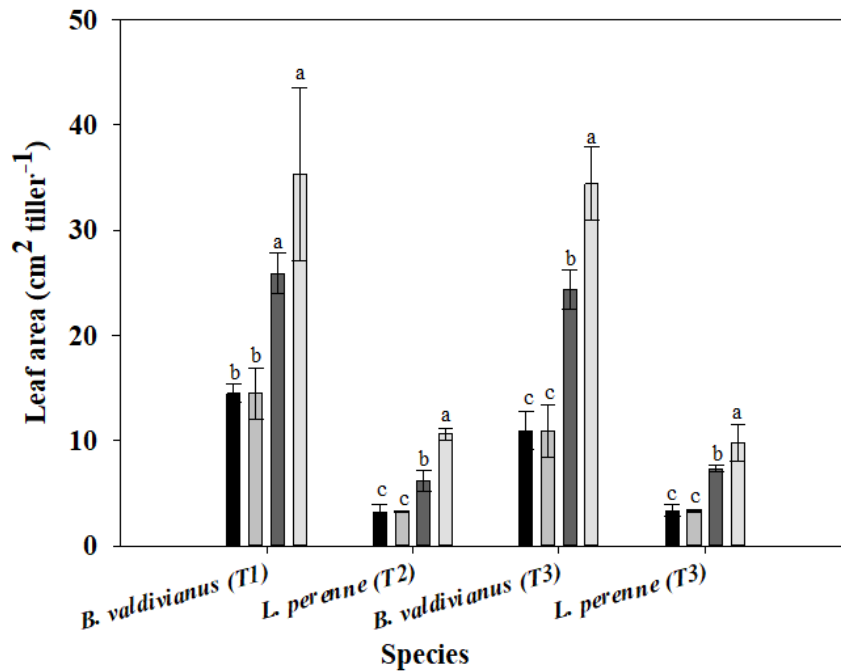


Figure 5.5 Leaf area per tiller at different leaf stages. *Bromus valdivianus* in T1 (in monoculture), *B. valdivianus* in T3 (in mixture), *L. perenne* in T2 (in monoculture) and *L. perenne* in T3 (in mixture). Columns indicate different leaf stage (LS) for, *B. valdivianus*: 1.75 LS (■); 2.75 LS (▒); 3.5 LS (▓); 4.0 LS (□), and for *L. perenne* 1.75 LS (■); 2.25 LS (▒); 2.6 LS (▓); 3.5 LS (□). Bars indicate standard error of the mean (n=15). Lowercase letters indicate significant differences between leaf stages.

The tiller population was correlated with environmental variables, such as air temperature, soil temperature at 10 cm soil depth, soil water content at 20 cm, rainfall and radiation (Table 5.3). The tiller populations of *B. valdivianus* in monoculture and in mixture were positively correlated to SWC, air temperature and soil temperature, but

negatively correlated to radiation. In contrast, the tiller population of *L. perenne* in monoculture was highly correlated to SWC, but negatively correlated to air temperature and soil temperature. The tiller population of *L. perenne* in the mixture was only strongly correlated to radiation.

5.3.3 Tiller leaf area and weight at different leaf stage development

Figure 5.5 shows leaf area for *L. perenne* and *B. valdivianus* at different leaf regrowth stages during March and May 2018. *Bromus valdivianus* tillers had a higher leaf area ($p \leq 0.001$) and a higher tiller weight ($p \leq 0.001$) compared to *L. perenne* tillers, at all regrowth stages and treatments. In relation to the comparison between monocultures and mixtures within species no differences were found ($p > 0.05$). On average, in this evaluation, *B. valdivianus* had 3.8 times higher leaf area per tiller (cm^2) than *L. perenne*. These results are in line with the results from core sampling, which indicated that *B. valdivianus* had 3.3 times more leaf area per tiller than *L. perenne* (Table 3.1).

5.3.4 Relative competition indices

The relative competition index (RCI) of *B. valdivianus* and *L. perenne* are shown in Figure 5.6A. The RCI were similar at the start of the evaluation period, March 2017, until October 2017, prior the first water restriction period (Figure 5.3). The RCI for both species diminished to values close to 0 during the water restriction period in December, indicating a similar effect of intraspecific and interspecific competition on the evaluated parameter, however, when the values became negative it indicated that intraspecific competition was more relevant to the outcome of competition, a situation reported for *L. perenne* (T2) between December 2017 and April 2018. The latter was due to the higher relative tiller population of *L. perenne* in the mixture than in the monoculture. However, after the water restriction of December, *B. valdivianus* increased its RCI, indicating a

higher competition intensity and reached values between 0.39 and 0.69, showing the effect of *L. perenne* over *B. valdivianus* in tiller population. The *L. perenne* RCI after the water restriction period fluctuated between -0.19 (08/01/2018) and 0.18 (30/05/2018), suggesting that is a better competitor than *B. valdivianus*.

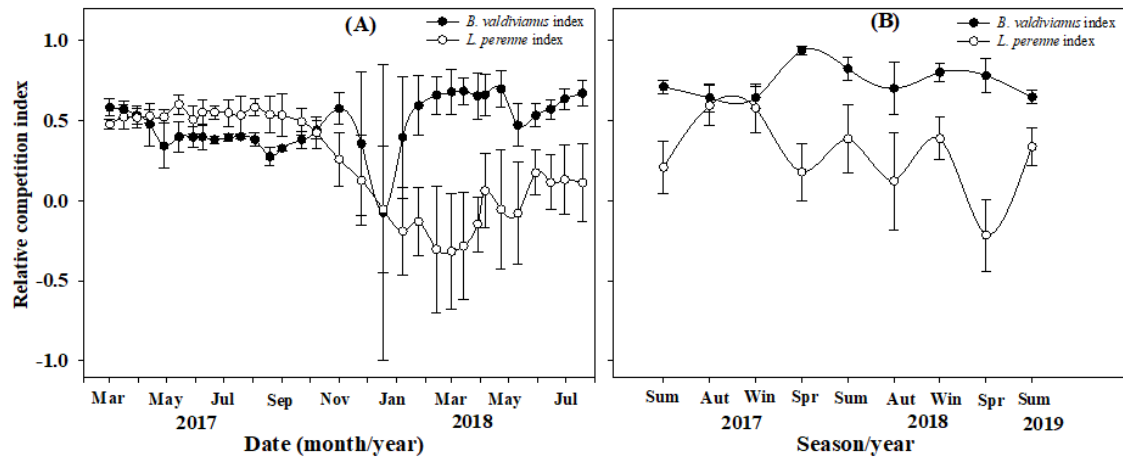


Figure 5.6 Relative competition indices for the tiller population (A) and for the seasonal herbage mass (B) for *B. valdivianus* and *L. perenne* during the evaluation period. Bars indicate standard error of the mean, for A (n=3) and B (n=4).

In Figure 5.6B, *L. perenne* had a lower RCI than *B. valdivianus* in all seasons apart from autumn and winter 2017, suggesting a better performance under competition than *B. valdivianus*. When *L. perenne* reached values close to 0 which meant that at that time the performance was similar between the monoculture and the mixture. Even though *L. perenne* always had a lower RCI (Figure 5.6B).

5.3.5 Estimated leaf area index and photosynthesis carbon fixation capacity

The photosynthesis carbon fixation capacity (PCFC) is in function of LAI (Figure 5.7A) and net photosynthesis (described in the methodology). Values for LAI and PCFC reach of 0 or close to 0, indicate that a grazing would have occurred close to that date (Figure

4.2). After the grazing occurred, the increasing PCFP is related to the increasing LAI of the pasture. Therefore, the maximum LAI measured in both species was reached at pre-defoliation (mean \pm sem); 2.7 (\pm 0.7) for *B. valdivianus* monoculture (13/04/2017), 3.0 (\pm 0.4) for *L. perenne* monoculture (22/09/2017); 1.5 (\pm 0.1) for *B. valdivianus* in the mixture (22/09/2017); 2.4 (\pm 0.3) for *L. perenne* in the mixture (08/01/2018); and 3.0 (\pm 0.1) for the *B. valdivianus* + *L. perenne* mixture (T3) as a whole (22/09/2017).

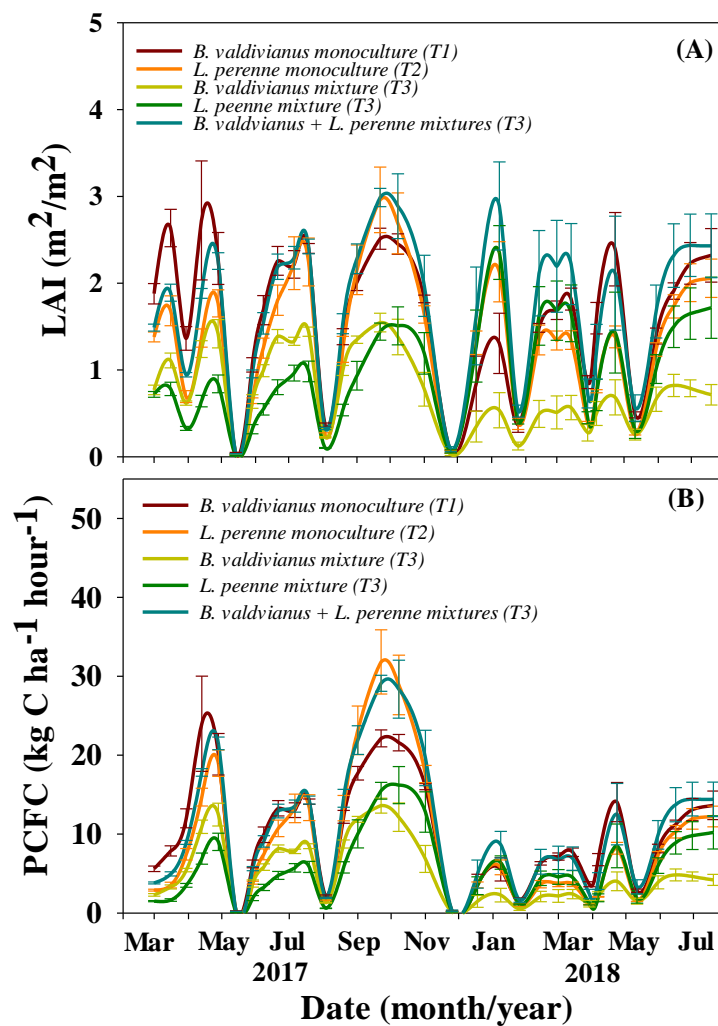


Figure 5.7 Estimation of the leaf area index (LAI) (A) and total carbon fixation capacity (PCFC) (B) for *B. valdivianus* in T1 and T3, *L. perenne* in T1 and T3 and for the total tiller population of T3 (sum of *B. valdivianus* in T3 + *L. perenne* in T3). Bars indicate standard error of the mean (n=3).

This analysis will be focus on the species LAI (Figure 5.7A). The mixed pasture, *B. valdivianus* + *L. perenne* (T3), tended to have the maximum values during almost all the evaluation period in comparison to *B. valdivianus* (T1) and *L. perenne* (T2) which were negatively affected during their critical periods, water restriction for *L. perenne*, and waterlogging and probable loss of functional roots for *B. valdivianus*. The diminishment of LAI during the winter and early water restriction period (November 2017 – December 2017) for *B. valdivianus* (T1) diminished its PCFC (Figure 5.7B) and *L. perenne* (T1) diminished its LAI during water restriction periods, therefore decreasing the PCFC (Figure 5.7B).

For PCFC, the peaks of each species and treatment will be analysed. The PCFC included the carbon transformed in biomass (above and below ground), the carbon losses by respiration and the release of organic compounds. *Bromus valdivianus* (T1) had a higher PCFC (mean \pm sem) at the beginning of the evaluation period, between 01/03/2017 and 13/04/2017, and reached a maximum PCFC of 24.0 kg C ha⁻¹ hour⁻¹ (\pm 6.0), followed by *B. valdivianus* + *L. perenne* (T3), and *L. perenne* (T2). Between the 14/05/2017 and 21/06/2017, *B. valdivianus* monoculture (T1) and T3 (*B. valdivianus* + *L. perenne* mixture) had significantly higher PCFC (mean \pm sem) than *L. perenne* monoculture (T2). This trend changed between the 3/08/2017 - 08/10/2017 where *L. perenne* monoculture (T2) and *B. valdivianus* + *L. perenne* mixture (T3) were the treatments with the higher PCFC (mean \pm sem) having a maximum of 31.8 kg C ha⁻¹ hour⁻¹ (\pm 4.1) and 29.1 kg C ha⁻¹ hour⁻¹ (\pm 1.0). This pattern indicates that the PCFC values diminished for *L. perenne* during the water restriction periods and for *B. valdivianus* after the waterlogging due to the loss of tillers and therefore the loss of functional roots. Thus, the *B. valdivianus* + *L. perenne* mixture (T3) tended to have higher numbers during all the critical periods due to a compensation growth between the two species.

When the *B. valdivianus* + *L. perenne* mixture (T3) was analysed as separate species, it was observed that *B. valdivianus* had a higher PCFC than *L. perenne* from the start of the evaluation until the 22/09/2018, where PCFC shifted between the species. The higher values for *L. perenne* were maintained during almost all the following measurement dates, reaching maximum values of 16.2 kg C ha⁻¹ hour⁻¹ (± 2.4) on 08/10/2017.

5.4 Discussion

5.4.1 Tiller population dynamics

Leaf regrowth stage is a measure of physiological development for grasses, and reflects the water soluble carbohydrate (WSC) reserve levels of individual tillers, and so can be used as a defoliation criterion for grasses (Fulkerson and Slack, 1994a,b; Donaghy and Fulkerson, 1997,1998; Ordóñez et al., 2017; Chapter 3). For *B. valdivianus*, the leaf regrowth stage to optimise shoot growth rate, tiller population, production and survival has previously been determined to be at around leaf stage 4 (Ordóñez et al., 2017; Chapter 3), which in a temperate environment is equivalent to *L. perenne* leaf stage of 3.5 (Chapter 3). The WSC reserves for both species, at those leaf regrowth stages, have recovered post-defoliation to levels close to those prior to defoliation (Donaghy and Fulkerson, 1997,1998; Ordóñez et al., 2017; Chapter 3). Therefore, the tiller population dynamics results from the interaction between climatic conditions, grazing events (Figure 4.2) and the competition between the species.

The relationship between tiller appearance rate, leaf appearance rate and leaf elongation duration has been reported by Allard et al. (1991) and Bahmani et al. (2000). The probability of a tiller bud developing into a tiller is evaluated as the proportion between the number of tillers in relation to the number of leaves, is called 'site filling' (Davies, 1974), afterwards tiller survival, in high productive pastures, will depend on the

environmental conditions (Mathew et al., 2000). *Bromus valdivianus* showed a phyllochron of 94°C day, which was slightly faster than *L. perenne* (Chapter 3). However, *B. valdivianus* needs to accumulate more growing degree days to complete the leaf elongation (Chapter 3; Ordóñez et al., 2017). Over all grazing events (Figure 4.2), *B. valdivianus* was defoliated with 6 leaves (leaf stage 4) and *L. perenne* with 4 leaves (leaf stage 3.5; Chapter 3). *Bromus valdivianus* tiller population was less dense than *L. perenne*, suggesting that *B. valdivianus* had a lower site filling than *L. perenne*.

All the morphological and architectural traits can be modified by the environment (Nelson, 2000) and management (Mathew et al., 2000; Hernández-Garay et al., 1997b), however, it is known that the plasticity traits (e.g. site filling) are species-specific (Huber et al., 1999). Therefore, the changes due to environment conditions are defined by the genetics of each species. Diggle (1994) reported that tiller growth has different responses due to the canopy removal and the changes in light properties (red/far red relationship). Gautier et al. (1999) reported an increase in tiller number when the main axis increased the quantity of fully expanded leaves. Also, there is a change in the site filling according to grazing and no grazing conditions, and therefore the light properties.

Bahmani et al., (2000) showed differences related to site filling between cultivars for *L. perenne* under shade and no shade treatments. Thus, post-grazing biomass is an important variable that affects the site filling of the species. Genetically improving a species by choosing an ecotype with higher site filling traits may be a good strategy to improve tiller populations and above ground production. However, from the point of view of resource allocation, if the plants of a single species, are selected just considering their higher site filling values, the ecotypes chosen could decrease the root exploration due to a prioritization of resources towards the shoot.

Soil moisture (Davies and Thomas, 1983; Bahmani et al., 2000; Matthew et al., 2000), light (Allard et al., 1991; Kays and Harper, 1994; Bahmani et al., 2000; Matthew et al., 2000) and temperature (Bahmani et al., 2000; Matthew et al., 2000) are environmental variables involved in tiller emergence and survival. However, it has been suggested that the site filling of a species might be independent of the environmental conditions (Davies and Thomas, 1983).

The effect of environmental conditions (e.g. soil moisture, soil temperature) over tiller population, is a relationship that is hard to assess, because all environmental parameters are related to each other (Davies and Thomas, 1983). For example, the increasing soil temperature, from 8°C (July) to 24°C (December), produced a negative effect on tiller populations ($p \leq 0.01$). However, it must be clear, that soil thermic properties are modified by the soil water content (Tyson et al., 2001), in such way that they are inversely proportional. In the present study the minimum soil temperature reached (7.5°C) did not restrict tiller population growth, indicating that the soil temperature was not a limiting variable to tiller appearance. However, tiller appearance strongly responds to soil water content, especially during spring and summer periods, when water become a limiting factor for both, pasture and tiller population growth diminished.

High temperatures tend to reduce tillering through a reduction in plant WSC reserves (Matthew et al., 2000) and occur in late spring and summer periods, in which soil water restriction is also a common factor. One of the causes of the decline in tillering is the low WSC reserves due to high temperatures causing higher plant respiration during the day and night (Fulkerson and Donaghy, 2001) and also is linked to the plant's capacity and ability to fix enough carbon to maintain its development during stress periods (e.g. waterlogging as well as water restriction). Therefore, it is expected that plant

physiological response to soil water restriction or waterlogging would differ according to each species, due to their own specific traits. For example, species for which growth is negatively affected by high temperatures and low SWC are species with reduced access to soil water storage, e.g. *L. perenne* (Crush et al., 2005; Ordonez et al., 2018), *T. repens* (Chapter 4), and *Agrostis capillaris* (Crush et al., 2005). In the present study, the tiller population of *L. perenne* was highly affected by the high temperatures (Matthew et al., 2000) in interaction with low SWC, probably by a diminishment of the WSC, possibly by its low soil exploration capacity (Figure 5.3A; Chapter 4). Contrarily, for *B. valdivianus*, tiller population was recovered during the dry summer due to its higher soil exploration capacity, supported by the SWC values at 40 cm soil depth during summer and its tiller population increase during the same period, between December and March (Figure 5.3).

Hernández-Garay et al. (1997b) showed a large decrease in tiller population during winter, from 15000-20000 tillers per m², down to 10000-15000 tillers per m² (in fixed quadrats). In contrary, the present study showed a constant increase in *L. perenne* relative tiller population during the winter period, which could have being related to the grazing criteria applied allowing sufficient WSC to accumulate in the stubble to support tiller population growth (Donaghy and Fulkerson, 1997,1998; Volaire and Lilievre, 1997; Fulkerson and Donaghy, 2001).

Plant survival from waterlogging is greater when WSC concentration is high (Jurczyk et al., 2016). In the present study, the constant increase in *L. perenne* tiller population under waterlogging also suggests that it has a high tolerance to low soil oxygen content (McFarlane et al., 2003). The high WSC concentration (Chapter 3) could explain *B. valdivianus*' survival and its tiller population stability during the waterlogging periods.

However, waterlogging had negative effects on *B. valdivianus* tiller population which was verified when the waterlogging period ended. The sudden change in environmental conditions from waterlogging (winter until early October 2017) to a drought period (November 2017 - December 2017), in around 4 weeks after the waterlogging ended, may have caused a functional root decrease, due to the low soil oxygen content (Chapter 4). The effect of waterlogging on the functional roots has been reported for several species including *Bromus catharticus* (Ploschuk et al., 2017), *L. perenne* (McFarlane et al., 2003), *Dactylis glomerata* (Etherington, 1984; Ploschuk et al., 2017), and *Festuca arundinacea* (Ploschuk et al., 2017). Stewart (1996) indicated that *B. valdivianus* also has a low tolerance to a lack of oxygen in the soil due to waterlogging. This suggests that *B. valdivianus* may have lost functional roots due to waterlogging during the winter, explaining the decrease in tiller population during the following water restriction (November 2017 – December 2017). The decreased *B. valdivianus* functional roots is confirmed with the SWC data between October 2017 and December 2017, where the SWC of *B. valdivianus* (T1) at 40 cm was similar to *L. perenne* (T2) suggesting the same water uptake. However, during the period that the *B. valdivianus* tiller population started to recover (January 2018 – March 2018), the SWC was the lowest, suggesting a higher soil water uptake suggesting a recovering of functional roots, and as a consequence of this the recovering of its tiller population. Therefore, during the first water restriction period (between November 2017 - December 2017), *B. valdivianus* lost the competitive ability provided by its deeper root system, but accomplished a recovery, starting during the second water restriction period (end of January 2018 – March 2018).

Regarding *L. perenne* (T2), its lower water uptake at 40 cm soil depth (derived from SWC data) could explain the high tiller mortality during the first water restriction period

(November 2017 – December 2017) and the second restriction period (end of January 2018 - March 2018), showing it to be an unstable species during water restriction periods.

Tiller mortality and appearance rate are associated with climatic variation, grazing (Bullock et al., 1994) and reproductive development (Hernández-Garay et al., 1997b). When *B. valdivianus* tiller populations decreased by half (between November and December) light competition may have diminished.

Thus, the increase of *L. perenne* (T3) could have been due to an interaction of, bare soil colonisation and the decrease in light competition. During that period, *L. perenne* tillering capacity was encouraged by the low tiller density of the pasture (Marriot et al., 1997), resulting in a significant *L. perenne* tiller population increase, reaching higher relative values than *L. perenne* (T2), changing the plant community structure (Crawley and May, 1987; Tilman et al., 1988). This increase in *L. perenne* tiller population in T3 affected the Pearson correlation. This does not mean that *L. perenne* (T3) was non-dependent of environmental conditions or grazing, it just indicate that the *L. perenne* tiller populations could have been closely related to the tiller density diminishment of the pasture (Bullock et al., 1994) and encouraged by the light reaching *L. perenne* buds. This resulted in a *L. perenne* relative tiller population increase from 200% to 350% in two weeks, but also with a significant decline between the soil water restriction period between end of January 2019 - March 2019.

Reproductive stage in grasses generates a diminishment in tiller population (Hernández-Garay et al., 1997b; Nelson, 2000) and the appearance of new tillers stops (Nelson, 2000). It has been reported tiller mortality during the reproductive stage, before seed head removal (Hernández-Garay et al., 1997b). Have been suggested that this could be in relation to hormonal changes (Clifford and Langer, 1975), formation of the inflorescence

(Clifford, 1977) and a change in carbon distribution within the plant (Ryle, 1970). The stem extension in *L. perenne* may reduce the growth of tillers and roots because the photoassimilates could be absorbed by the stem, becoming the stem the major sink (Ryle, 1970).

However, the sink of the resources changes again towards the vegetative tillers during the last stages of seed production (Hampton et al., 1987). Furthermore, the flowering development in grasses could be an important variable for the post-flowering tillering in *L. perenne*, due to the carbon movement from the flowering towards the daughter tillers (Matthew, 2002). During the reproductive stage, lax grazing followed by a hard grazing led to an increase in tiller population (Xia et al., 1990). Therefore, heading date of the species and cultivar, and the grazing management during the reproductive stage are important variables to increase the number of daughter tillers during summer/autumn seasons.

Lolium perenne cv. Rohan has a late heading (Agriseeds, 2014). *Bromus valdivianus* has a medium late heading (Stewart, 1996). In Southern Chile, according to IDE (1996), the heading is between November – January. The cultivar Bareno (*B. valdivianus*) has been described as a cultivar with a heading of +19 days after than the cultivar Gala (*B. stamineus*), an early heading cultivar (Agriseeds, 2013). Descriptions that for both species matches with a reproductive stage around late spring - beginning of summer. Therefore, if it is considering the low frequency of the grazing management (4.0 LS for *B. valdivianus*; 3.5 LS for *L. perenne*), the grazing realized in late spring 24/11/2017 (Figure 4.2) and the sharp increase of *L. perenne* tiller population between December - January, the mobilisation of resource described by Matthew (2002) probably was a mechanism involved. This could contribute to explain the major mortality of tillers during summer,

since daughter tillers did not have had the proper water supply conditions to survive during summer due to the soil reaching permanent wilting point.

As *B. valdivianus* and the cultivar Bareno have been described as a mid-late heading, the sharp decrease between October-December could have been a late consequence of the to the waterlogging over the loss of functional roots during the winter rather than the reproductive stage. In mid-December, three weeks after the last grazing (24/11/2017) *B. valdivianus* started to recover its tiller population, however it seems that was more related to an increase in the soil water uptake at 40 soil cm depth. Either way the parameters related to the environmental conditions, cultivar development stage and agricultural management must be considered in tiller population dynamics. For future research for *B. valdivianus*, it must be considered a separation of vegetative and reproductive tillers to determine the reproductive tiller replacement and if resources mobilisation mechanism described for *L. perenne* (Matthew ,2002) is also happening for *B. valdivianus*.

5.4.2 Tiller growth strategy

The integration of data provided by the cores (Table 5.1) and the individual tillers harvested (Figure 5.5) determined that *B. valdivianus* tiller leaf area, at a leaf stage 3.5 - 4.0, is at least 3.3 times higher than *L. perenne* at a leaf stage 3.0 - 3.5, but had a lower tiller population.

The results obtained from the SWC may indicated that a deeper soil root system could be an indication of a high photo-assimilates allocation for *B. valdivianus* root system in comparison to *L. perenne*, however this needs to be quantified. Given the higher *B. valdivianus* photosynthesis per tiller due its greater leaf area and longer leaf lifespan (Chapter 3), the amount of resources allocated per root should be higher than *L. perenne*. This would explain why *B. valdivianus* had a higher water uptake at the deep soil layers

compared to *L. perenne* (T1) after its recovery. Therefore, the results of the present study suggest that the growth strategy of *B. valdivianus* is focused on biomass production per tiller (including both, shoot and root) with a few numbers of them, contrasting with *L. perenne*, which is centred on small tillers, with a faster tiller appearance getting a greater tiller population. These attributes of *L. perenne* allow it to rapidly colonise empty spaces in the pasture (Marriot et al., 1997), under the favourable conditions, temperature and non-limited water supply compared to *B. valdivianus* (Figure 5.3; Figure 5.4; Chapter 4). However, this faster tillering may have a consequence of a lower allocation of photo-assimilates to the root system, making the species highly sensitive to water restriction, and the latter was reflected in the high mortality rates during the two periods of water restriction (Figure 5.3).

The morphological traits of *B. valdivianus* and *L. perenne* (Chapter 3; López et al., 2013), along with their growth strategy (Chapter 3) and root exploration traits (Chapter 4; Ordóñez et al., 2018), allow them to uptake resources from different soil ecological niches, allowing them to share the same pasture ecosystem, as has been observed in the south of Chile (López et al., 1997).

5.4.3 Competitive ability

In an ecosystem where resources are limited, their uptake and utilisation by plants are key factors determining competition outcome (Lehman and Tilman, 2000; DeClerck et al., 2006). The uptake rate of resources, the level of environmental stress and ecosystem perturbation did not vary the intensity of the competitive interaction, but changed the dynamics of the ecosystem and the outcome of the competitive process between species (Tilman, 1988; Grace, 1995). In the present study, waterlogging followed by soil water restriction negatively affected growth traits of *B. valdivianus*, and consequently its

competitive ability (e.g. death of functional roots) which observed by the low water uptake at 40 cm soil depth (higher SWC content) between October 2018 - mid December 2018).

The RCI evaluated the competition by comparing the performance of the target species in intraspecific and interspecific competition. Values close to 0 it means that the intra and interspecific competition had the same effect over the target species' growth. Values close to 1 means that the target species is highly affected by the interspecific competition produced by the other species. Negative values mean that intraspecific competition is more significant than the interspecific competition. The RCI reached zero during December 2017 indicating that for both species had similar values in both, intra and interspecific competition, therefore, there was no competition effect related to tiller populations between *L. perenne* and *B. valdivianus*. When the waterlogging conditions followed by the water restriction period occurred, *L. perenne* kept values close to 0 while *B. valdivianus* RCI increased, showing how the environmental stress affected the outcome of the competition between both species (Tilman, 1988) and how *L. perenne*, under this study conditions, was a better competitor than *B. valdivianus* in both indices, tiller population and herbage mass per season. Thus, the competition with *B. valdivianus* did not affect the variation of the tiller population for *L. perenne*.

Within the competitive ability of a plant, two concepts can be distinguished: 1) the plant's capacity to resist the suppression wielded by other plant species, and 2) the plant's capacity to suppress growth of other plants (Golberg and Landa, 1996; Golberg, 1996). The results presented in Figure 5.6 showed how *L. perenne* had a great capacity to suppress the growth of *B. valdivianus*, maybe due to the loss of functional roots, as previously mentioned. Although, apparently *B. valdivianus* did not suppress *L. perenne* growth, it did

show a competitive response against the suppression capacity of *L. perenne*, this is supported by the constant increase in population in both, *B. valdivianus* in T1 and T3 from January until the end of experimental period. As a result of this, by the end of the summer drought period the tiller population of *B. valdivianus* had almost recovered its original status; *B. valdivianus* in T1 reached 106% and T3 reached 88% in comparison to the original population.

5.4.4 Carbon fixation capacity

The estimation of PCFC considered *B. valdivianus* in T1 and in the T3, *L. perenne* in T2 and in T3 and the pasture mixture (T3) considering *B. valdivianus* plus *L. perenne*. Although the contribution by other species (e.g. white clover, other grasses and weeds) was not considered, both measured species were dominant in their respective treatments (see botanical composition in Chapter 4), consequently the results reflected the PCFC of each species. The objective of these calculations was to compare both species in respect to PCFC.

Grazing and fertilization affect the allocation of photo-assimilates (Wang et al., 2007). For example, under grazing conditions, after 10 days of plant growth, plant respiration was about 50 - 70% of the carbon fixation due to photosynthesis, allocated about 13 - 25% of the carbon towards the shoots and 18 - 40% towards the roots (Wang et al., 2007; Pausch and Kuzyakov, 2018). Although, from the present results, it is possible to hypothesise that the differences in above-ground biomass could be due to differences between carbon allocation patterns, considering of course that main driver of carbon fixation is the light interception. Chapter 4 showed how *L. perenne* tended to have a higher accumulated herbage mass during the whole first year of evaluation. Therefore, it is highly probable there were differences related to photo-assimilated allocations patterns

in both species. Figure 5.7B shows that *B. valdivianus* (T1) had higher PCFC than *L. perenne* (T2) at specific dates, which should have been reflected in the accumulated herbage mass production or growth rate (Chapter 4), but it was not.

Poorter et al. (1994) reported that species with higher transpiration rates and low water use efficiency are correlated with higher root carbon allocation, which could be also associated with longer root length. This makes sense, since a high transpiration rate is highly correlated to a higher soil water availability, and therefore, a deeper soil exploration by roots (Chapter 4; Blum, 2009). The allocation of carbon between the plant structures (e.g. roots vs shoots) changes the function of the plant species' growth strategy (Poorter et al., 1994, 2012; Siebenka et al., 2015). A higher root carbon allocation by *B. valdivianus* would explain its higher capacity for water extraction from deep soil layers, higher than *P. lanceolata* at 70 cm (Chapter 4). Siebenkäs et al. (2015) indicated that the functional group of tall grasses, including *Dactylis glomerata*, had a higher allocation of carbon towards the root system. Currently, most defoliation management only considers above-ground biomass production, with no consideration of the total pasture ecosystem biomass production (shoots plus roots), which is an important parameter (root) to include to reach sustainable management of pastures in climates with dry seasons. Therefore, this estimation raises the question regarding the differences in photo-assimilate allocation between these two species, and it can be hypothesised that *B. valdivianus* allocated a higher percentage and quantity of its photo-assimilated towards its roots than *L. perenne*.

The diversity-stability hypothesis states that a higher species diversity brings stability to an ecosystem (Elton, 1958; Tilman and Downing, 1994; Hooper and Vitousek, 1997; Tilman, 1999; Isbell et al., 2009). However, functional diversity (Hooper and Vitousek, 1997; Tilman, 1999; Hooper and Duke, 2004; Tilman et al., 2006; Isbell, 2009), different

ecosystem niche exploration (Trenbath, 1974; Hooper and Vitousek, 1997; Hooper, 1998; Loreau and Hector, 2001; Hooper and Duke, 2004) and growth asynchrony (Loreau, 2010; Thibaut and Connolly, 2013; Zhang et al., 2018) have recently become regarded as more relevant than simply 'species richness'. It seems that *B. valdivianus* + *L. perenne* (T3), in relation to PCFC and LAI, responded better to critical periods due to its higher functional diversity, where niche differentiation played an important role. This is visible during the peaks before every grazing and is a result of by their physiological and morphological differences in shoot growth and root development (Chapter 3; Chapter 4; López et al., 2013. Ordóñez et al., 2017). When all these attributes are considered together, such as niche differentiation evaluated through their soil water uptake (Chapter 4), photosynthesis carbon fixation capacity PCFC and LAI of the mixed pasture (T3) is the one that presented higher values in each critical period. For example, PCFC was higher in the mixture (T3) than in the T1 and T2 after the waterlogging occurred (October 2017 - December 2017) negatively affecting *B. valdivianus* growth, and during summer period (December 2017 - February 2018) negatively affecting *L. perenne* growth. Even if the stability was not evaluated, growth compensation was observed. When two species hit their critical periods, one diminished, while the other increased its presence in the pasture, consistent with the stability-diversity theory (Tilman, 1999), creating more stable production systems under environmental constraints.

The evaluations of soil water dynamics gave an insight of the different mechanisms for water uptake of *B. valdivianus* and *L. perenne*. There are several other factors that need to be studied to gain a better understanding of the interaction between species and environment, and to make inferences regarding mixes versus monocultures, such as how the different root traits affect soil pore continuity (Peth et al., 2008; Peth et al., 2010; Uteau et al., 2013), and therefore the water redistribution and water lifting (Richards and

Coldwell, 1987; Coldwell and Richards, 1991; Coldwell et al., 1998; Skinner, 2004; Skinner et al., 2009; Chapter 4). Also the allocation differences of the photo assimilate ,i.e. between grass species (Poorter et al., 1994, 2012) and pasture management (Wang et al., 2017), is an important variable to consider to create more sustainable productive ecosystems, because it would help to understand how to manipulate the dynamic of photo assimilate distribution between shoot and root, through the pasture management.

5.5 Conclusions

Bromus valdivianus is a species that grows and develops better than *L. perenne* under soil water restriction, but it is sensitive to waterlogging. *Bromus valdivianus* tolerates the suppression exerted by *L. perenne* development especially during the soil water restriction period.

Lolium perenne, based on its higher tillering capacity, colonises pasture gaps left by other species and dominates the pasture during periods when other species are growing slowly, seen in the T3 treatment. Due to its shallow soil exploration, *L. perenne* is sensitive to soil water restriction, resulting in declining tiller numbers over summer.

Lolium perenne tiller population was shown to be highly resilient, and during favourable conditions related to temperature and soil moisture overcomes *B. valdivianus* tiller population. *Bromus valdivianus* higher leaf area and root exploration (derived from WSC data) allowed it to successfully compete and recover itself when it was competing with *L. perenne*.

The mixture of *B. valdivianus* and *L. perenne* (T3) was shown to be more stable during the study period for all the evaluated parameters than were the individual species as

monocultures. The mixture had a higher PCFC and tiller population during all the critical periods, such as the waterlogging and soil water restriction periods.

Chapter 6 General discussion

6.1 The current challenge for pastoral agriculture

Sustainability in pastoral agricultural systems is an important objective, due to increasing food demand and environmental issues related to intensive use of ecosystem resources (Butler and Soskolne, 2013), where intensive use of natural resources and land management results in negative ecosystem effects (Leng et al., 2014; Gigon, 1983; Wild and Cameron, 1980; OECD, 1982; Ellies, 1988; Di and Cameron, 2002; Cameron et al., 2013; Butler and Soskolne, 2013; Li et al., 2017), with practices that exceed the ecosystem services that the land and environment can cope with (Millennium Ecosystem Assessment, 2003).

Current traditional agricultural managements and practices must be challenged to secure sustainable production for the future. One of the strategies to reach sustainability is the development of more stable agricultural productive systems (Patzek et al., 2008; Ulanowicz et al., 2009) and one way to do it, is by increasing plant diversity within pastoral agricultural ecosystems (Tilman, 1999; Hooper and Vitousek, 1997) together with a suitable defoliation interval (Fulkerson and Donaghy, 1998), both being the main drivers of this doctoral thesis.

6.2 The main differences between *B. valdivianus*, *L. perenne* and *P. lanceolata*

Hooper and Vitousek (1997) stated that to understand the species behaviour according to their own traits, it is necessary to design experiments that compare species under conditions of both monoculture and mixture, in order to not confuse the results with other variables, e.g. interspecific competition. That approach was considered for the current research, where *B. valdivianus*, *L. perenne* and *P. lanceolata* were compared both as

monocultures and as mixtures and the subsequent changes in the botanical composition within the pasture through time.

The morphological traits observed in Chapter 3, 4 and 5, tiller number, weight, photosynthetic carbon fixation capacity, root growth (through soil water uptake), leaf area, number of leaves and leaf expansion indicated that *B. valdivianus* has a different growth strategy to that of *L. perenne*, being the growth and survival of single tillers, with more number of leaves, each with higher leaf area and a deep root system, known indirectly by the soil water content measured from the deep soil layers, compared with the production of multiple tillers, each with smaller leaf area and a shallow root system for *L. perenne*. Even though *L. perenne* was a stronger competitor in the mixtures than *B. valdivianus* (T4 and T5), due to greater resilience after the environmental stress.

Bromus valdivianus produced more photoassimilates per tiller (Chapter 3 and 5), and prioritised the allocation of resources to the leaf area production and root system, with a greater tiller survival, tiller appearance rate and growth during the periods of water restriction (Chapter 3 and 5). Contrary to what was expected, *P. lanceolata* extracted less water than *B. valdivianus* at 70 cm soil depth, but throughout the evaluation period, *P. lanceolata* maintained a high plant contribution, over 70% of botanical composition in the monoculture pasture (T3 in Chapter 4) and becoming the dominant species in the full mix pasture (T5 in Chapter 4) over *L. perenne*, proving itself to be a good competitor in a pastures mixtures.

As water restriction progressed, the major physiological response in species was observed, with *L. perenne*, a species with a relative shallow distribution of roots, exhibiting lower rates of photosynthesis, higher osmotic potential and lower stomatal conductance than *B. valdivianus* and *P. lanceolata*. However, high levels of osmotic

potential are associated with increased water uptake when soil water tension is high (Chimenti et al., 2006; Blum 2009). This poses the question of whether the main reason for *L. perenne* having a high osmotic potential is driven more by genetics, or by its relatively shallow root distribution in the soil. It was also observed that *P. lanceolata* had the lowest stomatal conductance, and this was maintained during conditions of high temperature with low water availability, even though *P. lanceolata* increased its herbage mass during periods of water restriction, however, the higher photosynthesis and higher stomatal conductance could also be linked to a low stomata control in comparison to *L. perenne* and *B. valdivianus*. For example, differences related to the morphology and speed reaction of different stomata structures have been assessed, being the graminoid-type (*Triticum aestivum* L.) with a faster reaction (opening and closing). This could be explained by differences in the geometry of the cell guard and the osmotic shuttling (Fran and Farquhar, 2007).

6.3 Why do complex productive ecosystems reach higher stability and sustainability?

Figure 2.1 illustrates that to reach sustainable production, it is necessary to forego maximum production, and in return the ecosystem gains resilience and complexity through a higher functional diversity (Patsek et al., 2008). The complexity of an ecosystem is related to both biotic (e.g. plant species) and abiotic factors (e.g. soil water content), as well as the interactions between them (Hooper and Duke, 2004). For example, an increase in soil pore continuity due to root traits (Peth et al., 2008, 2010; 2008; Uteau et al., 2013), the relationship between below-ground biomass production and soil resilience following mechanical stress (i.e. trampling; Zúñiga et al., 2015; Ivelic-Saéz et al., 2015; Ordóñez et al., 2018), and an increase in water uptake due to pasture diversity

increased (Blum, 2009; Ordóñez et al., 2018), or the possible water lifting realised by the deep-rooted species (Richards and Coldwell, 1987; Caldwell and Richards, 1989; Skinner et al., 2008; Chapter 5).

DeClerk et al. (2016) and Tilman and Downing (1994) indicated that an ecosystem resists environmental stress if the dominant species is resistant to that stress, with the result that herbage mass production will not be greatly affected. This was verified in the current study, where *P. lanceolata* increased herbage mass during summer and *L. perenne* increased herbage mass during winter and spring within the mixture (T5 in Chapter 4). A similar result was obtained for *B. valdivianus*; increased herbage mass during summer and increased tiller population in both monoculture and mixture with *L. perenne* (Chapter 4 and Chapter 5). Therefore, if pasture mixes are designed to blend species with tolerance towards different critical climatic periods, then an exchange of dominance between species is likely to occur across the seasons, providing stability in herbage mass, and resulting in a more complex ecosystem, which is one of the important goals for achieving sustainability (Patzek et al., 2008; Ulanowicz et al., 2009).

The interaction between biotic and abiotic factors was identified, through evaluating plant-related parameters (e.g. productive, morphological, and physiological parameters) and soil water dynamics. Changes in soil water content indirectly reflected the quantity of functional roots and the water uptake by plants, and a decrease in soil water content between species was associated with a higher effectiveness in water absorption (Blum, 2009; Neal et al., 2012; Ordóñez et al., 2018), considering that evaporation was equal for all treatments. The T5 mixed pasture in Chapter 4, dominated by *P. lanceolata* and *L. perenne*, displayed the highest growth rates and the highest water uptake at 40 cm soil depth during water restriction periods, and at 70 cm soil depth, the water uptake almost

matched that of the most effective treatments T1 and T3 (Chapter 4), which were both monocultures, *B. valdivianus* and *P. lanceolata*, respectively. These monocultures were characterized by a greater composition of deep-rooted species compared to the T5 mixture (Chapter 4). On the other hand, during winter, the T5 mixed pasture had a higher water uptake than the T1, T2 and T3 monocultures, due to a high proportion of *L. perenne* (Chapter 4). These results are consistent with those of Ordóñez et al. (2018), suggesting that higher pasture diversity had a greater water uptake at 60 cm (due to the presence of deep-rooted species) and *L. perenne* had a higher water uptake at 10 cm soil depth. This greater water uptake can be explained by the exploration of different ecological niches (Hooper and Duke, 2004). For example, species with a deeper root distribution explore niches where the water is protected from evaporation, and this enhances production during periods of water restriction as was observed in Chapter 4 and Chapter 5. Furthermore, Chapter 5 showed that as *B. valdivianus* regained its potentiality for water uptake at 40 cm depth (may due to functional roots recovery), shown by the middle of December 2018. This recovery was reflected by its constant tiller population increase during the summer. On the other hand, incorporating species with tolerance to waterlogging (e.g. *L. perenne*), even if they have less tolerance to water restriction, helps to enhance herbage production during winter.

Even though water restriction is a major stress that affects productive ecosystems, simply evaluating water dynamics and pasture stability is not enough to determine the sustainability of the productive system. This is because there are changes in the pastoral ecosystem generated by management and biodiversity, which it may include a redistribution of water (Richards and Coldwell, 1987; Coldwell and Richards, 1991) due to the presence of deep-rooted species within the pasture, where inconclusive data were generated in Chapter 4, a soil porosity interconnection between the surface soil and the

deep soil layers may be found (Peth et al., 2008, 2010), an increase in functional diversity generating a positive effect in ecosystem food webs (Reiss et al., 2009; Lavorel et al., 2013) and a change in plant photoassimilate distribution due to a longer defoliation interval (Donaghy and Fulkerson, 1998; Chapter 3). Many factors need to be included in pastoral science if the aim is to create a model to accomplish sustainable production systems.

6.4 The key to reach sustainability, diversity-stability hypothesis

The differences between species related to physiology, morphology and growth strategy were the factors that influence asynchrony and portfolio effect (Tilman, 1999; Hooper and Duke, 2004; Loreau and de Mazancourt, 2008; Isbell et al., 2009) in the present thesis. The higher leaf area and lower tiller number of *B. valdivianus* compared to *L. perenne* (Chapter 3 and 5) or the probable differences in their root life span (Van Der Krift and Berendse, 2002; Watson et al., 2000) and photoassimilates distribution (Poorter and Farquhar, 1994; Chapter 4 and 5), may explain the differences for their growth rate, survival and recovery after the environmental stress. For *P. lanceolata*, the interaction between a higher temperature requirement (Lee et al., 2015) and deeper root traits (Chapter 4) determined its growth rate during water restriction. For trait differences constitute the elements that lead to niche differentiation, such as space differentiation (different root depth) and time differentiation (different growing rates and therefore different resources uptake rates; Hooper and Duke, 2004).

Thus, the presence of species with different critical growth periods diminished the effect of seasonality and weather on the pasture growth rates, especially in T5 (the full mixture in Chapter 4). The latter was aligned with Hooper and Vitousek (1997) and Hooper and Duke (2004) that reported that different functional groups explain ecosystem stability,

rather than the functional richness by itself. Therefore, it can be suggested that the ecosystem stabilising mechanisms could be driven mainly by the functional traits of the species and grazing management. This statement is supported by the results of Chapter 4 and Chapter 5.

6.5 Gaps behind the theory of diversity-stability

The concepts previously discussed (portfolio effect, asynchrony andoveryielding) reported by Tilman (1999), Hooper and Duke (2004), Tilman et al. (2006), Isbell et al. (2009) and Yang et al. (2011), came from long-term experiments based on the annual accumulated herbage mass, where plants were defoliated using a lawnmower once per year. This situation is far removed from practical grazing management. The direct effect of grazing animals on soil and pasture species communities, combined with multiple seasonal defoliations per year and the defoliation criterion applied, plays an important role in the growth dynamic of pasture species, along with species succession and grazing selection of individual plants or plant parts (Perotti et al., 2018; Grace et al., 2019), nutrient incorporation through urine (Di and Cameron, 2002; Cameron et al., 2013), and dung (Jarvis et al., 1989), trampling effect (Ellies et al., 1988; Dörner et al., 2013), and change of carbon allocation due to defoliation and fertiliser addition (Wang et al., 2007). Also, the regrowth and development of pasture species is modified by the opportunity of each particular defoliation event in relation to the physiological development of each individual plant (Alberda, 1966; White, 1973; Donaghy and Fulkerson, 1997, 1998; Turner et al., 2006a, b, c, d; Turner et al., 2007a, b; Ordóñez et al., 2017; Chapter 3) and the intensity or height of defoliation (Fulkerson and Donaghy, 1998), all these factors interact affecting the species persistence, survival and its proportion in the pasture. None of these important pastoral ecosystem variables were considered in the studies of Hooper

and Vitousek (1997), Hooper and Duke (2004), Tilman et al. (2006), Loreau et al. (2001) and Isbell et al. (2009).

DeClerck et al. (2006) reported that a greater diversity in a pine tree ecosystem (up to 4 different pine tree species: *Pinus contorta* ssp. *Murrayana*, *Pinus monticola*, *Abies magnifica*, and *Tsuga mertensiana*), tended to both decrease the ecosystem resistance under soil water restriction, and increase the coefficient of variance, failing to prove that higher diversity brings a higher stability (the portfolio effect). The significant role of ecosystem stability and functioning was driven by the presence of stress-tolerant or stress-sensitive species. Therefore, to create ecosystems that are resistant to environmental constraints, stress-tolerant species must dominate the ecosystem (Lehman and Tilman 2000; DeClerck et al., 2006). DeClerck et al. (2006) hypothesised that two different mechanisms drive the ecosystem resilience and resistance: 1) resource partitioning (niche partitioning), positively related to diversity; and 2) competition for limiting resources, where the dominant species should be more tolerant than the other species to the environmental constraint levels, an attribute that is negatively related to the diversity effect (portfolio effect).

In the present study, the effectiveness of water uptake from the treatment co-dominated by *P. lanceolata* and *L. perenne* (T5 in Chapter 4) provided higher herbage mass throughout the water restriction periods, although there were no differences in the annual accumulated herbage mass. Thus, ecosystem resilience and resistance, as major factors to attain stability, are driven by the specific environmental variables that constitute a constraint to plant survival or growth. In order to build complex productive systems that are resistant and resilient to stresses, the interactions between ecosystem attributes, species traits and defoliation management must be understood. In this study, resistance

and resilience during periods of soil water stress (waterlogging and soil water restriction; see Chapter 4 and 5), were driven by the species traits that allowed tolerance of water restriction (e.g. root depth, osmotic potential), waterlogging (e.g. aerenchyma), physiological features (e.g. optimum growth temperatures), morphological features (e.g. root size, leaf area) and the longer interval between grazing.

Bromus valdivianus, *L. perenne* (Chapter 4 and 5) and *P. lanceolata* (Chapter 4) were shown to be highly competitive, with a good survival and recoveries after environmental stress. Chapter 5 explored how a mixture between two grasses achieved stability regarding photosynthetic carbon fixation capacity and tiller population. Regarding to photosynthetic carbon fixation capacity, was shown differences between *L. perenne* and *B. valdivianus*, therefore it can be hypothesized differences related to photoassimilates distribution throughout the plant, considering that the accumulated herbage mass was the same for all the treatments.

In previous research, also in a temperate humid climate (Valdivia, Chile), it was shown how *L. perenne* water uptake was mainly from the first 10 cm of the soil depth, under a defoliation criterion of the 2.5-leaf stage (Ordóñez et al., 2018). In the present thesis, *L. perenne* (T2 in Chapter 4) was not the treatment with higher water uptake at 10 cm soil depth, as in Ordóñez et al. (2018), it is possible that the root system of *L. perenne* was deeper in response to the interaction of both a longer defoliation interval [3.5 leaf stage in the current study, compared to 2.5 leaf stage used by Ordóñez et al. (2018)] and intraspecific competition, allowing *L. perenne* to explore a deeper soil layer, between 20 and 30 cm soil depth.

Grazing alters pasture components and soil attributes, and needs to be considered when overyielding and portfolio effects may affect ecosystem stability (Tilman et al., 1997;

Tilman, 1999; Isbell et al., 2009). However, overyielding has not yet been reported in a grazing system with perennial species (Flores et al.; 2017; Ordóñez et al., 2018; Grace et al. 2019; Chapter 4). In contrast, a negative effect on the herbage mass of a mixed pasture has been reported, due to plant selection by grazing animals, which detrimentally affected growth in the selected species, breaking the synergic effect between species, and resulting in decreased herbage mass produced by the mixture compared to the monocultures (Grace et al., 2019). This was also reported by Flores et al. (2017), Ordóñez et al. (2018) and in Chapter 4, where no statistical differences were found related to accumulated herbage mass between the species pasture evaluated. Also, an increase in plant diversity is not always related to an overyielding (Flores et al., 2017; Ordóñez et al., 2018) or a greater ecosystem stability such as soil mechanical resistance and resilience (Zúñiga et al., 2015; Ivelic-Saéz et al., 2015; Ordóñez et al., 2018).

The mechanism described by Tilman (1999) and Isbell et al. (2009) explained the pasture ecosystem stability under different grades of plant diversity, although this has not been tested in long-term pasture experiments, which would include multiple grazing events during the year along with fertiliser application. In the present thesis, less fluctuation of herbage mass was recorded, deduction that is born from the growth rates obtained during critical periods in comparison to monocultures, however, no overyielding was obtained.

6.6 Reconciling intensive production systems with sustainability: greater ecosystem stability at low diversity

For decades it has been recognised that intensive pastoral grazing systems are positively correlated with a production of greater herbage mass (Hudson, 1929; Gammon, 1978; Hernández-Garay et al., 1997a,b; Holecheck et al, 1999; Donaghy and Fulkerson, 1998), within some boundaries to agricultural managements imposed by nature, for example

post-grazing pasture defoliation height (Donaghy and Fulkerson, 1998). Most of the research undertaken regarding the efficiency of the grazing systems has been based on biomass production and the defoliation criteria applied, either according to the above-ground herbage mass or time based. Thus, the carbon fixation of plants, both above and below ground, in field conditions under grazing with different defoliation frequencies, has not been evaluated, and it has not been determined if grass plants change their carbon allocation according to the pastoral management (Wang et al., 2007). Chapter 3 results showed that leaf stage 4 was defined as the best criterion to encourage *B. valdivianus* regrowth, equivalent to the 3.5-leaf stage for *L. perenne* and overcoming the AGDD needed by *P. lanceolata* to encourage its regrowth, i.e. 532 °C days (Lee et al., 2015). *Bromus valdivianus* defoliation criterion allowed them to recover the WSC reserves and was applied to all the treatments in Chapters 4 and 5, decision that was based by the criterion of WSC and above ground plant parameters for the herbs and grasses.

Incorporating root biomass production as a pasture management parameter could lead to the development of defoliation criteria to achieve the highest total biomass production (root plus shoot), which would increase the resilience of the pastoral system when subjected to water stress. Therefore, as a first approach can be hypothesised, from the results obtained in Chapter 3 and from the Chapter 4, that longer grazing interval will enhance the growth of the root system, improving the species survival during soil water restrictions.

Lower defoliation frequency did not reduce the final pasture yield, according to the literature (Hernández-Garay et al., 1997a), and could have been the reason for the high species survival (see botanical composition in Chapter 4) and great tiller population recovery after environmental stress (Chapter 5), due to a higher WSC levels (Chapter 3

and 5). However, several questions could be raised from the effects of the longer intervals of defoliation, such as the effect on species survival, persistence and phenotypic plasticity. For example, *L. perenne* and *B. valdivianus* tiller population dynamics (Chapter 5); may be assume that the longer interval of defoliation was an important variable that allowed them to recover tiller populations after the stress for both species. Or the fact that *L. perenne* in other studies showed clearly a greater water uptake at 10 cm soil depth in field condition in comparison to others species (Ordóñez et al., 2018), and considering to the data presented in this thesis, that result was not observed, raising the question of whether such differences in root depth are mainly caused by climatic and soil conditions, or by the management applied. In fact, when defoliation interval was longer (see Chapter 3) the root growth was higher, therefore, it can be assumed a similar behaviour for *L. perenne* and *P. lanceolata*.

Bromus valdivianus and *P. lanceolata* are species with deep soil exploration and a fast root development, resulting in higher water uptake at 70 cm soil depth [i.e. T1 (*B. valdivianus*) > T3 (*P. lanceolata*) > T5 (*B. valdivianus* + *L. perenne* + *P. lanceolata*); Chapter 5]. The reason of the shallow root traits of *L. perenne* could be linked to its short period of life (Watson et al., 2000), having a root death up to 90% after 42 days (Van Der Krift and Berendse, 2002), or a root life span for *L. perenne* of 15 weeks, the lowest values in comparison to other species, such as, *Molinia caerulea*, *Arrhenatherum elatius*, *Nordus stricta* for example.

6.7 The future of water supply on productive systems

There are three sources of water supply for agricultural activity, 1) water from local rainfall, 2) surface water such as rivers, lakes, wetlands and groundwater, 3) non-renewable groundwater and non-local water resources (Vörösmarty et al., 2005).

Recently research in hydrology indicates that irrigation systems are unsustainable especially in light of global warming (Gleeson et al., 2010; Wada et al., 2012; Leng et al., 2015). Ground water supply utilisation has tripled between 1960 – 2000 (Wada et al., 2012). If groundwater sources recharge is less than the groundwater extraction over extensive periods of time, groundwater depletion will occur (Gleeson et al., 2010).

Pastoral agricultural systems must be aligned to the ecosystem services at a local scale, in order to reach sustainability considering both biotic and abiotic factors. One strategy that can contribute to the water scarcity is to integrate ecological theory into agricultural system management, and in this manner the efficiency of the resource utilisation can be maximised, however this does not mean that production will increase (Patzek et al., 2008), but that it will be more stable and therefore, sustainable. In this sense the results of the present thesis have shown the value of increasing functional diversity in pasture species used in grazing systems by mixing shallow-rooted species with deep-rooted species, to achieve greater tolerance of critical soil water stress periods through increasing the effectiveness of soil water uptake (Blum, 2009) and through the inclusion of species tolerant to waterlogging, the two major stresses identified during this study, using grazing interval that allow the full replenish of WSC in all the species involve within the pasture. Therefore, the development of more complex ecosystems, considering the interaction and integration of functional diversity, resource utilisation and production efficiency, are the key in this novel approach to reach long-term sustainability of pastoral systems.

Chapter 7 Conclusions

Key conclusions regarding individual species were:

The defoliation criterion at 4-leaf stage was determined to be the appropriate one to enhance *B. valdivianus* above ground growth and its non-structural carbohydrates storage. Coincidentally, the defoliation frequency based on *B. valdivianus* at 4-leaf stage had a positive effect on the production, stress survival and recovery of itself, *L. perenne* and *P. lanceolata*. Waterlogging during the winter months negatively impacted *B. valdivianus*, decreasing tiller population, functional roots and its contribution to the pasture botanical composition. Defoliating *B. valdivianus* at the 4-leaf stage resulted in a recovery after waterlogging to close to its initial population. *Lolium perenne* dominated the monoculture and the mixture with *B. valdivianus*, only decreasing its tiller population during the water restriction periods, while *P. lanceolata* maintained and dominated the botanical composition when sown as a monoculture and as a mixture with either *B. valdivianus* and *L. perenne*.

Although *B. valdivianus* displayed physiological and water uptake adaptations to resist the water restriction, it has a low tolerance to winter, i.e. waterlogging. In contrast, *L. perenne* has low resistance to water restriction, but greater resilience than *B. valdivianus* when the environmental conditions favour its development.

Key conclusions regarding to the increase of the functional diversity within the pasture were:

Combining species with both a shallow and a deep distribution of roots increased the functional diversity in pasture and resulted in a higher production of herbage mass during the majority of the seasons evaluated (5 of 9), particularly during periods of restricted soil

water, compared to monoculture pastures. Such a pasture can be considered as highly stable.

In the diverse pastures, the species contribution varied throughout the year according to the environmental factor that was limiting plant growth, such that *P. lanceolata* dominated the diverse pasture during the summer drought periods and co-dominated with *L. perenne* during the wetter and colder winter-spring.

Mixing species according to complementary root and physiological traits (*P. lanceolata*, *L. perenne* and *B. valdivianus*) and environmental stress tolerance, and then exploring optimal defoliation ‘windows’ to determine a period of overlap that encourages the regrowth of all species in the mixture, was shown to be a feasible strategy to promote persistence while maintaining stable herbage mass, resulting in a more balanced and stable pastoral system.

Independent of the differences found related to the physiological and morphological parameters of the species, or the differences related to the botanical composition and water uptake between monocultures and mixtures pastures, no treatment differences were found in annual accumulated herbage mass.

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Appendices

Appendix 1 General view of the *B. valdivianus* leaf stage trial described in the experimental chapter 3.



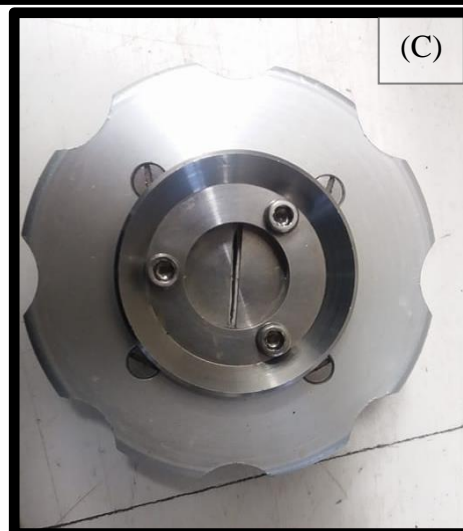
Appendix 2 Distribution of the plants per pot as described in the experimental chapter 3.



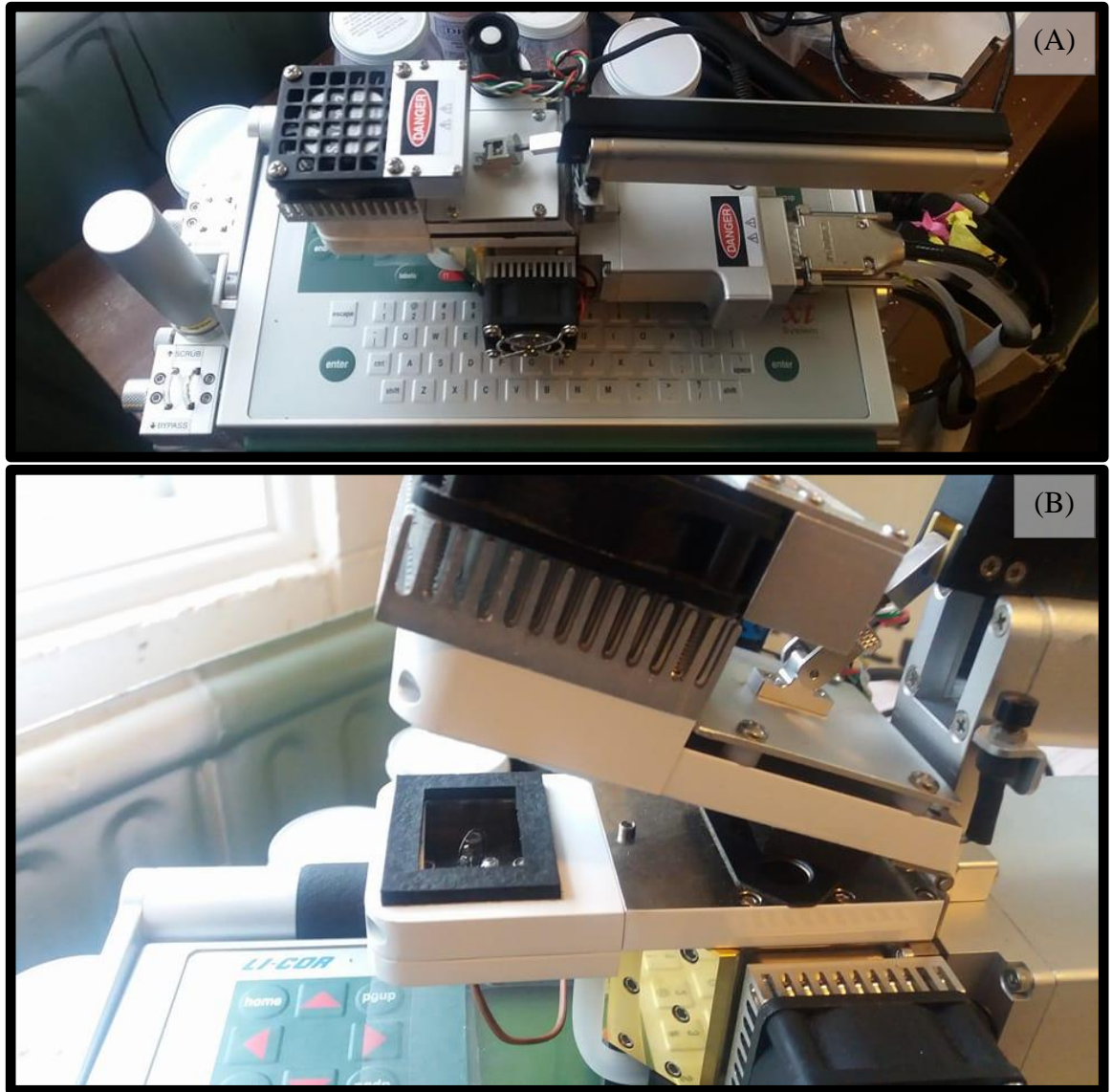
Appendix 3 Pastures under evaluation as described in the experimental chapter 4 and 5.



Appendix 4 Field set up for the leaf water potential and osmotic potential evaluations as described in experimental chapter 4 (A). Close up of the Scholander pressure bomb and its lid (B y C).



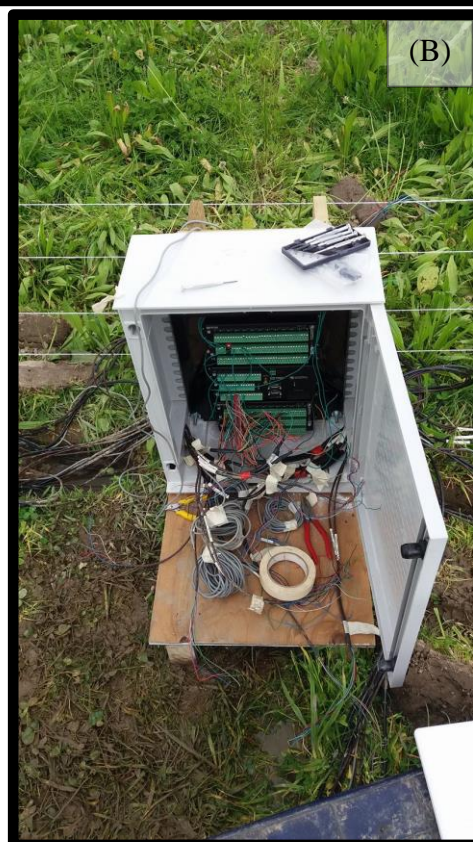
Appendix 5 General views of the LICOR-6400XT (LI-COR, Nebraska, USA; A) and the chamber 6400-02B LED light source (LI-COR, Nebraska, USA; B) used for the photosynthesis evaluations as described in the experimental chapters 3,4 and 5.



Appendix 6 General view of the HR-33T Dew Point Microvoltmeter (Wescor, Logan, Utah, USA; A) and the C-52 chamber (Wescor, Logan, Utah, USA; B) used for the osmotic potential evaluations as described in the experimental chapter 4.



Appendix 7 Close up of the CE-8 Excitation Module (Campbell scientific, Logan, Utah, USA), CR1000 data logger (Campbell scientific, Logan, Utah, USA) and 16/32B multiplexer (Campbell scientific, Logan, Utah, USA) used for the evaluation of soil water content and soil matric potential as described in the experimental chapter 4 and 5 (A). General view of the field set up as described in experimental chapter 4 and 5 (B).



Appendix 8 Setting up of the sensors (CS-616 and 229) at 10 and 40 cm soil depth as described in the chapter 4 and 5.



Appendix 9 Portable metallic rings (A, B and C) demarking fixed areas to determine tiller number as described in the experimental chapter 5.

