



Morpho-physiological and productive adjustments of *Lolium perenne* L. and *Agrostis capillaris* L. under incident light diminishment

Ajustes morfo-fisiológicos de *Lolium perenne* L. y *Agrostis capillaris* L. como respuesta a la disminución de la luz incidente

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ARTICLE INFO

Keywords:

Grasses species
Tiller
Photosynthetic active radiation
Chlorophyll
Shade

Original Research Article,
Special Issue: Agroecology and
Sustainable Agricultural Systems

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ABSTRACT

Lolium perenne L. (Lp) and *Agrostis capillaris* L. (Ac) are perennial grasses and there is a lack of information regarding their growth strategies when incident light becomes limited. The objective was to evaluate morpho-physiological and productive responses of Lp and Ac with incident light diminishment. Three incident light regimes were imposed regarding photosynthetic active radiation (PAR): 60.7, 140.5 and 199.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, named Heavy Shading (HSh), Medium Shading (MSh) and Full Sunlight (FS), respectively. The pasture used was a humid temperate naturalized pasture, with (FP) and without (NFP) fertilizer addition, being Lp and Ac targeted. Metal rings were placed in the pastures under each shading condition and Lp and Ac growth evaluated. The experimental design was a randomized complete block with factorial arrangement of the treatments: three levels of shading (HSh, MSh and FS), two levels of fertilization (NFP, FP) and two species (ring class: 100% Lp tillers and 100% Ac tillers); in 3 blocks. It was concluded that in a restricted soil nutrient condition (NFP), Lp DM accumulation and tiller density were greater in MSh and HSh than FS, whereas in contrast Ac DM yield and tiller density were greatest in FS. Crude protein, chlorophyll content index, leaf area and leaf length for Lp were greatest in FP-HSh and for Ac lowest in NFP-FS. MSh and HSh created an environmental condition with enough WC, N and PAR to favor pasture production, a fact that implies the mitigation of the drought stress effects in the future climate change scenario.

RESUMEN

Lolium perenne L. (Lp) y *Agrostis capillaris* L. (Ac) son especies perennes en las cuales poco se conoce de las estrategias de crecimiento luz incidente es limitante. El objetivo fue evaluar los cambios morfo-fisiológicos de Lp y Ac frente a la disminución de la luz incidente. Tres niveles de luz incidente fueron aplicados basándose en la radiación fotosintéticamente activa (PAR): 60,7, 140,5 and 199,9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, siendo denominados Sombreamiento Alto (HSh), Sombreamiento Medio (MSh) y expuesto a la luz (FS), respectivamente. La pradera utilizada fue una pradera naturalizada sin fertilizar (NFP) y otra fertilizada (FP). Argollas de metal fueron colocadas en cada pradera y en sombreamiento que contenían 100% Lp y 100% Ac. El diseño experimental fue de 3 bloques completos al azar con un arreglo factorial de los tratamientos. Se concluyó que cuando la restricción de nutrientes en el suelo fue baja (FP), DM y densidad de macollos de Lp aumentó bajo MSh y HSh, mientras que en NFP, fue Ac quien incrementó DM y densidad de macollos en FS. Al aumentar el sombreamiento incrementó la proteína cruda, índice del contenido de clorofila, área foliar y largo de lámina, donde Lp tuvo los valores más altos en FP HSh y Ac los más bajos en NFP FS. MSh y HSh crearon una condición ambiental con suficiente WC, N y PAR para favorecer la producción de la pradera, un hecho que implica la mitigación de los efectos del estrés por la sequía en el futuro escenario de cambio climático.

Palabras clave: Especies de gramíneas, macollos, radiación fotosintéticamente activa, clorofila, sombra.

INTRODUCTION

The environment has a strong influence on pasture growth and on practices to reach sustainable agricultural systems. Soil nutrients, water, temperature and radiation are recognized as main factors regulating pasture growth (Kwon and Woo, 2016), and pasture improvement processes to increase annual pasture herbage mass accumulation towards its potential production (Tharmaraj *et al.*, 2008; Dekvota *et al.*, 2009; Sbrissia *et al.*, 2010; López *et al.*, 2013).

The intercepted light by a pasture canopy influences plant herbage mass accumulation (Zhu *et al.*, 2010). The light quantity (incident radiation) and light quality (light spectrum) affect pasture productivity and plant development (Chory, 2010; Kami *et al.*, 2010; Li *et al.*, 2012).

Morpho-physiological mechanisms of plant adaptation to light regimes suggests that the shade tolerance of species plays a relevant role in pastoral systems functioning (Valladares and Niinemets, 2008; Kwon and Woo, 2016). Shade tolerance indicates the capacity of plants to tolerate low light levels, with such an environmental constraint provoking a multifactorial plant attributes response (Martínez-García *et al.*, 2010). Shade tolerance in the future will become more relevant under global warming scenarios because it influences plant response to elevated temperatures and drought periods (Valladares and Niinemets, 2008; Kwon and Woo, 2016). The acclimation of plants to shade or sun conditions is accompanied by changes in the plant architecture, the content of pigments, leaf electron transport complexes and photosynthetic activity (Benkov *et al.*, 2008; Lichtenthaler *et al.*, 2013; Schöttler and Tóth, 2014). This could enhance the resilience of plants for protection against light stress and to withstand against fluctuations on soil water availability.

Plant adaptation to different light regimes can be influenced by environmental factors such as soil nutrient availability and grazing disturbance, and moderated by phenotypic plant plasticity (Valladares and Niinemets, 2008).

The most common studies regarding pastures species growing under different incident light conditions have been performed in silvopastoral systems, as consequence of a sustainable pastoral agricultural program, which implied tree plantation for soil conservation (Devkota *et al.*, 2009). In these systems, all pastures species decreased under tree shade being affected by the degree of competition between trees and pasture for light, moisture, and nutrients (Devkota *et al.*, 2009).

In contrast, other studies found a higher annual pasture accumulation herbage mass under moderate shade (Anderson and Moore 1987; Kyriazopoulos *et al.*, 1999), which reported that pasture production and its nutritive value in silvopastoral systems can be im-

proved introducing a shade tolerant grass (*Dactylis glomerata* L.) in mixture with legume species (*Trifolium subterraneum* L.), but the higher competitive ability of *D. glomerata* compared with *T. subterraneum* under shade should be taken into account (Kyriazopoulos *et al.*, 2013).

The pasture dry matter production is dependent on the radiation use efficiency and light interception, influenced pasture canopy height (Byrt, 2011). Shaded plants usually have thinner and wider leaves as well as higher specific area compared to unshaded plants (Wahl *et al.*, 2001; Zhai *et al.*, 2006). Leaf morphology is changed by phenotypic plasticity as a response to shading which could be a consequence of growth reduction due to resource limitation (da Silveira Pontes *et al.*, 2010). There are cases in which phenotypic plasticity increases plant production as an adaptation to environmental stress (Dingkuhn *et al.*, 2005) and the leaf elongation in response to shading is one of these (Flores, 2015). It has been suggested that the ability of plant species to shade other species within a community is the result of the interaction between genetic variation amongst population species and environmental factors (Abraham *et al.*, 2014). Differences in the response of grass species to shading in terms of plant growth, morphology and nutritive value have been reported in several studies (Dodd *et al.*, 2005; Devkota *et al.*, 2009; Cerovic *et al.*, 2012; Ehret *et al.*, 2015). However, there is limited information available regarding the effects of a range of incident light on pasture growth in grazing systems.

Lolium perenne L. (Lp) and *Agrostis capillaris* L. (Ac) are widespread perennial grass species, in Temperate Humid climates, which grow in a variety of environments. Neither species have been studied in terms incident light adaptation.

Herbage mass accumulation and leaf growth of *L. perenne* and *A. capillaris* under different incident light conditions may express different adaptive growth responses towards capturing light, which can affect *L. perenne* and *A. capillaris* colonization and succession in Temperate Humid climates in face of global change. The main objective was to evaluate dry matter production and leaf morphology of *L. perenne* and *A. capillaris* under different light intensities in natural degraded pastures.

METHODS

The study was carried out at Universidad Austral de Chile's Santa Rosa Research Station (39°47'26''S, 73°14'12''W), 9 km east of Valdivia city, Chile. The altitude is 25 m. a. s. l., with an historical average annual rainfall, calculated from rainfall records between 1901 and 2005, of 2442 mm (González-Reyes and Muñoz, 2013). The monthly rainfall and mean temperature va-

lues during the experimental period in Valdivia (October 2012-August 2013) are presented in Figure 1.

The soils are classified as Duric Hapludand, Valdivia series (IREN-UACH, 1978; CIREN, 2003).

The two pastures evaluated comprised a non fertilized naturalized pasture (NFP) and naturalized fertilized pasture (FP), layout in 400 m² plots. Each plot was grazed by 25 2-year-old sheep, Austral breed, live weight 55 ± 2.42 kg (average ± SEM) and body condition 2.71 ± 0.27 (average ± SEM, body condition scale from 1 to 5). The pastures were grazed when they reached an equivalent herbage mass of 2100-2300 kg DM ha. Each grazing event ended when the pasture herbage mass was down to 1000-1200 kg DM ha (Matthews *et al.*, 2004). If the pasture did not reach the pre-grazing mass criteria, the plots were grazed 60 days after the previous grazing event. Each treatment was independently grazed of the others according to the grazing targets used. Each grazing event lasted between half a day and one day.

Fertilizers were applied annually to achieve a pasture production of 9443 kg DM ha year⁻¹ in FP (80 kg N ha⁻¹ year⁻¹, 52 kg P ha⁻¹ year⁻¹, 100 kg K ha⁻¹ year⁻¹ and 2000 kg ha⁻¹ year⁻¹ of CaCO₃ as lime). NFP registered 5854 kg DM ha⁻¹ year⁻¹.

Soil samples were collected at the beginning of April 2012, before the fertilizer application. To determine soil nutrients status soil samples were taken from the top 20 cm of soil, from each NFP and FP plot (n= 3). One individual soil sample was analyzed for chemical status from each plot. Each individual soil sample was comprised of five soil subsamples. The methodologies applied to obtain the chemical status are described in Page *et al.* (1982), Sadzawka *et al.* (2006) and Van Reeuwijk (1986). They were used to determine: pH in water (pH_w), pH in calcium chloride (pHCaCl₂), soil or-

ganic matter (SOM), soil exchangeable potassium (K⁺), sodium (Na⁺), calcium (Ca²⁺), magnesium (Mg²⁺) and aluminum (Al³⁺), total soil exchangeable bases, sulphate (S-SO₄²⁻), soil cation exchange capacity and aluminum saturation (Alsat) as an index of the relationship between the amount of aluminum and the amount of total exchangeable bases plus the exchangeable aluminum, expressed as a percentage. Soil mineral nitrogen was determined as N-NO₃ and N-NH₄ using KCl (2 M) as extract, MgO, Devarda's alloy and distillation process, follow by H₂SO₄ (0.0025 M) titration (Bremner, 1996; Sadzawka *et al.*, 2006). Olsen-P was determined by extraction with sodium bicarbonate as NaHCO₃ (Olsen *et al.*, 1954). Soil total exchangeable bases (STEB) was calculated as described in Page *et al.* (1982) (Table 1).

In October 2012, the pasture botanical composition was determined in NFP and FP. Five pasture subsamples were taken from each plot using a stratified random method (Erickson and Nosanchuk, 1992; Steel *et al.*, 1997). The target sampling dates were when the seasonal growth and development of the pasture species were fully expressed, that was between the 1st and 15th of October 2012. The herbage was cut from a sampling area of 0.1 m² (quadrat) at ground level using the trim technique of Radcliffe (1974). The pastures species were manually separated and the proportion of each species contributing to the total dry matter was calculated. Relative abundance of species was calculated as the percentage of above ground biomass out of the total aboveground biomass per sample (Flores *et al.*, 2017).

NFP botanical composition constituted: Grasses e.g. 0.48% *Lolium perenne* L., 5.28% *Bromus valdivianus* Phil., 17.71% *Holcus lanatus* L., 1.30% *Dactylis glomerata* L., 0.24% *Schedonorus arundinaceus* (Schreb.) Dumort.; 34.46% *Agrostis capillaris* L., 6.17%

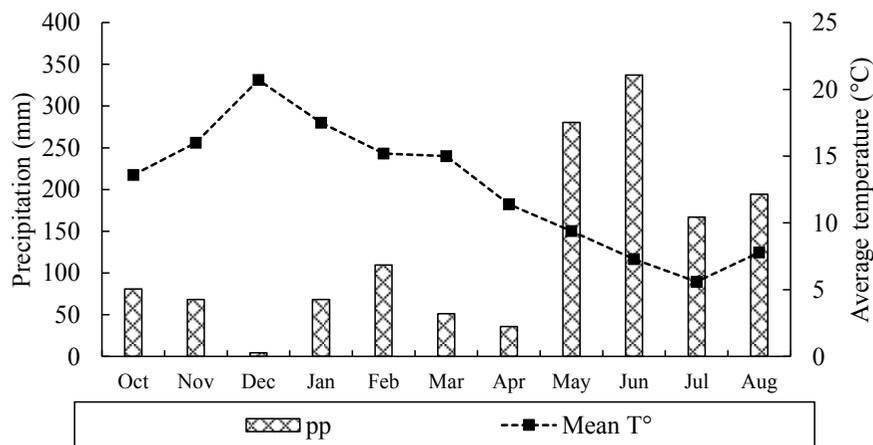


Figure 1. Meteorological conditions (pp, Mean T°) during the experimental period (October 2012-August 2013).

Figura 1. Condiciones meteorológicas (pp, T°Media) durante el período experimental (Octubre 2012-Agosto de 2013).

Table 1. Soil nutrients level. Soil samples were analysed on dry mass basis(Mean ± SE).

Cuadro 1. Nivel de nutrientes del suelo. Muestras de suelo fueron analizadas en base a materia seca (Media ± SE).

		FP	NFP	Significance
N	(mg kg ⁻¹)	88.7(±9.8) ^a	30.2(±1.4) ^b	**
Olsen-P	(mg kg ⁻¹)	11.45(±0.01)	9.43(±0.76)	ns
Exchangeable K ⁺	(cmol ⁺ kg ⁻¹)	0.35(±0.08) ^a	0.16(±0.01) ^b	**
Exchangeable Na ⁺	(cmol ⁺ kg ⁻¹)	0.22(±0.05) ^a	0.10(±0.01) ^b	**
Exchangeable Ca ²⁺	(cmol ⁺ kg ⁻¹)	6.41(±0.50) ^a	2.16(±0.13) ^b	***
Exchangeable Mg ²⁺	(cmol ⁺ kg ⁻¹)	0.97(±0.04) ^a	0.55(±0.05) ^b	**
Total Exchangeable Bases	(cmol ⁺ kg ⁻¹)	7.9(±0.5) ^a	2.9(±0.1) ^b	**
pH _w	(1:2.5)	5.85(±0.10)	5.63(±0.03)	ns
pH CaCl ₂	(1:2.5)	5.28(±0.09) ^a	4.92(±0.04) ^b	*
SOM	(%)	14.6(±0.3)	15.1(±0.4)	ns
S-SO ₄ ²⁻	(mg kg ⁻¹)	9.9(±0.2)	6.6(±0.7)	ns
Exchangeable Al ³⁺	(cmol ⁺ kg ⁻¹)	0.10(±0.02) ^b	0.43(±0.01) ^a	**
AlSat	(%)	1.6(±0.6) ^b	12.5(±0.6) ^a	***
CEC	(cmol ⁺ kg ⁻¹)	8.0(±0.4) ^a	3.4(±0.1) ^b	**

NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture; N, Soil mineral nitrogen; pH_w, pH in water; pH CaCl₂, pH in calcium chloride; SOM, soil organic matter; S-SO₄²⁻, sulphate; AlSat, soil aluminum saturation; CEC, cation exchange capacity. Different letters in each row for the same specie indicate significant differences (* P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001; ns P≥ 0.05)

Anthoxanthum odoratum L.; legumes e.g. 10.09% *Trifolium repens* L., 0.18 % *T. subterraneum* L., 0.3% *Lotus uliginosus* Schkur., and flatweeds e.g. 21.4% *Leontodon nudicaulis* L., and 2.30% *Taraxacum officinale* Weber.

While, FP was composed of grasses e.g. 7.94% *Lolium perenne* L., 4.1% *Bromus valdivianus* Phil., 22.2% *Holcus lanatus* L., 0.08% *Dactylis glomerata* L., 2.67% *Schedonorus arundinaceus* (Schreb.) Dumort.; 24% *Agrostis capillaris* L., 6.55% *Anthoxanthum odoratum* L.; legumes e.g. 5.24% *Trifolium repens* L., 0.06 % *T. subterraneum* L., 1.18% *Lotus uliginosus* Schkur., and flatweeds e.g. 16.8% *Leontodon nudicaulis* L., 2.07% *Hipochaeris radicata* L., 1.23% *Cirsium vulgare* L., 0.08% *Rumex acetocella* L. and 5.8% *Taraxacum officinale* Weber.

Within each plot, shading structures covers were used to reduce incident light to three levels of photosynthetic active radiation (PAR μmol m⁻² s⁻¹): 60.7, Heavy Shading (HSh; 30% PAR of sunlight); 140.5, Medium Shading (MSh; 70% PAR sunlight); and 199.9 Full Sunlight (FS). The structures dimension were as follow: 30 cm height, 70 cm length, 100 cm width for HSh and 20 cm height, 65 cm length, 75 cm width for MSh. The position of the shading structures was South-North and fixed during the whole experimental evaluation, except during the grazing events when they were moved out of the plot and placed back once the grazing finished. Each individual shading structure was cove-

red with polyethylene Raschel mesh, such that the desired PAR level was reached.

Soil volumetric water content (WC) of the top 10 cm soil depth (Dec et al., 2012) was measured every 15 days with time domain reflectometry (TDR) (Time Domain Reflectometry; TDR 200 Soil Multimeter FOM/mts, easy Test, Lublin, Poland.). WC was determined under each structure and measurements were taken between 11:00 h and 13:00. The photosynthetic active radiation (PAR), as photosynthetic photon flux intercepted in μmol m⁻² s⁻¹, was measured at ground level and above the pasture canopy, every 15 days with SKP 116 (400-700 nm; 600-800 nm) ‘Standard’ Fibre Optic Light Measuring System; Skye, Powys, UK. The PAR measurements were taken between 11:00 h and 13:00 h.

Pasture species measurements

The tiller density was measured using Bahmani et al. (2003) method modified. Under each shading structure, four metal rings (6 cm diameter) were placed in the pasture in a fixed position (October 2012). The rings (2 rings for each class) contained 100% *L. perenne* tillers (Lp), 100 % *A. capillaris* tillers (Ac). The number of live tillers within each ring was counted every 15 days. Within each ring, ten vegetative tillers were measured for leaf length (distance from the ligule to the tip of its lamina) every 15 days and prior each

grazing event. In the same leaves, chlorophyll content index (CCI) was determined using a digital chlorophyll meter CCM-200 Plus (OptiSciences, Tyngsboro, Mass.). The measurements were obtained from the adaxial leaf side facing the sunlight (Cerovic *et al.*, 2012). For each species and shading structure, individual tillers were cut at ground level ($n=100$) and collected, taken to a laboratory and their leaf area was measured using a leaf area meter (LI-3100C, LI-COR, Lincoln, NE, USA). Subsequently, each leaf was dried in an oven at 60 °C per 48 hr and weighed for dry matter (DM, mg) and for percent of crude protein. The percent of crude protein (CP) was measured on a Leco CNS Analyzer (Leco CNS-2000, St Joseph, Michigan, USA) which is based on the Dumas dry combustion technique. These measurements were performed once a season.

Statistical analysis

Treatments effects were analyzed according to a randomized complete block design with factorial arrangement of the treatments: 3 levels of shading (HSh, MSh and FS), 2 levels of fertilization (NFP, FP) and two species (ring class: 100% *L. perenne* tillers and 100% *A. capillaris* tillers). The analysis of variance (ANOVA) was used to determine statistical differences between treatments and LSD means comparisons applied to separate treatment means (Steel *et al.*, 1997). The relationship of tiller dry matter yield and shading with leaf

pasture species was estimated by regression analysis (Gianoli and González-Teuber, 2005).

RESULTS

Soil water content

The daily temperature and rainfall were measured under full sunlight conditions. The highest temperature and the lowest rainfall were registered in early summer (December) with an average daily temperature of 20.7 °C and 4.32 mm of monthly rainfall. In winter, the lowest temperature was registered in June and highest rainfall in July, with an average daily temperature of 5.6 °C and 337.3 mm of monthly rainfall (Figure 1).

Regarding the soil water content, the shaded treatments at all time (MSh and HSh) registered higher soil volumetric water content (WC) than FS in the first 10 cm of soil depth. This additional soil moisture content under shading structures resulted in a water recharge in winter, where FP HSh and FP MSh registered above. The lowest WC was registered in early summer (December) with an average 13.6% in FP and 14.8% in NFP (Figure 2).

Daily photosynthetic active radiation (PAR)

The daily photosynthetic active radiation (PAR) was integrated to calculate the accumulated monthly

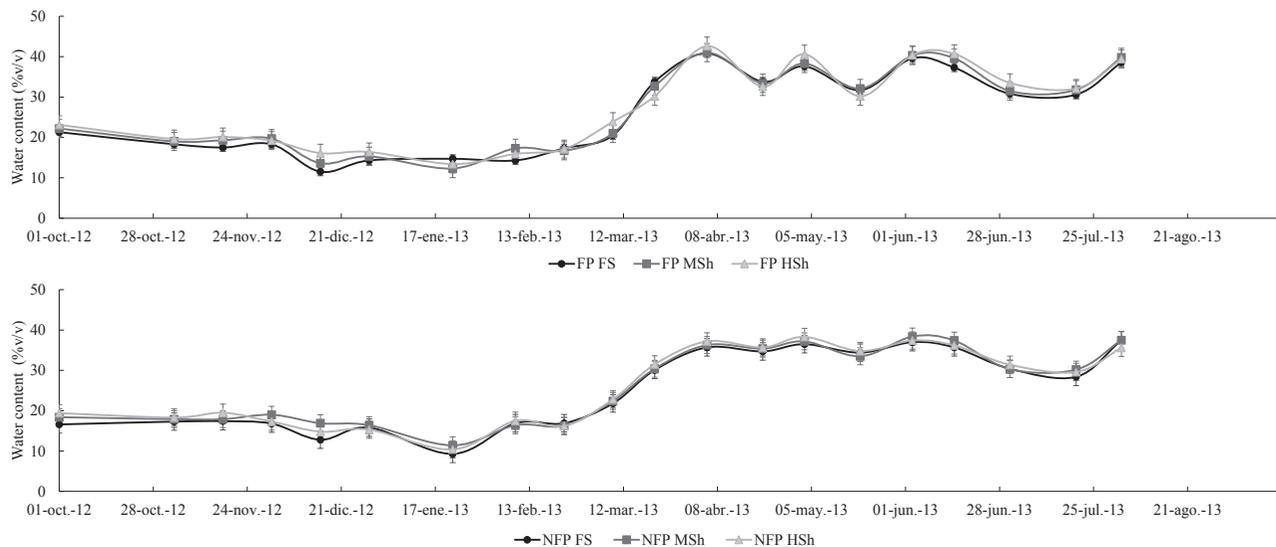


Figure 2. Soil water content at 10 cm of soil depth under two fertilizer regimes and three levels of shading. FP, naturalized fertilized pasture; NFP, non fertilized naturalized pasture; FS, Full sunlight; MSh, Medium shade; HSh, Heavy shade. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$.

Figura 2. Contenido de agua del suelo a 10 cm de profundidad bajo dos regímenes de fertilización y tres niveles de sombreado. FP, pradera naturalizada fertilizada; PFN, pradera naturalizada no fertilizada; FS, luz solar completa; MSh, sombra media; HSh, sombra alta*. $P \leq 0,05$; ** $P \leq 0,01$; *** $P \leq 0,001$; ns $P \geq 0,05$.

photosynthetic photons per unit area (Figure 3). The maximum photosynthetic photons reached in FP and NFP was in January for FS (189.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The minimum photosynthetic photons was in HSh in July (4.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

The daily PAR in the full sunlight for a sunny day in spring (solar angle elevation 53.4°), summer (solar angle elevation 67.4°), autumn (solar angle elevation 45.7°) and winter (solar angle elevation 32.2°) were used as reference to calculate transmissivity of the shading structures (Table 1). This was used to represent the relative reduction of photosynthetic photons in the shaded treatments compared with FS. The total amount of photosynthetic photons received in FS during summer (29th of December) was $183 \times 10 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was 4 times higher than in winter (20th July) (Table 2). The transmissivity on the shading structures decreased with a decline of the solar angle elevation from summer to winter.

Dry matter, tiller population density, crude protein, leaf length, leaf area and chlorophyll content index in each species

There were significant differences for shading level on Lp and Ac for DM, T, CP, LL, LA and CCI ($P \leq 0.05$). The CP, CCI, LL and LA increased as shading increased. The highest values of CP, CCI, LL and LA were registered in FP for HSh-Lp and the lowest in NFP for FS-Ac (Table 3 and Table 4). In FP, Lp had a higher tiller m^{-2} than Ac. In FP, Lp had a stronger tiller increase in MSh compared with Ac. In NFP, Ac tiller population density had the highest increase in FS and HSh (Table 3 and Table 4).

Lp had the highest values for DM and tiller density in FP (Table 5 and Table 6), while Ac had the highest values for DM and tiller density in NFP (during spring). The CP, CCI, LL and LA increased as soil nutrients levels increased, being registered the highest values for

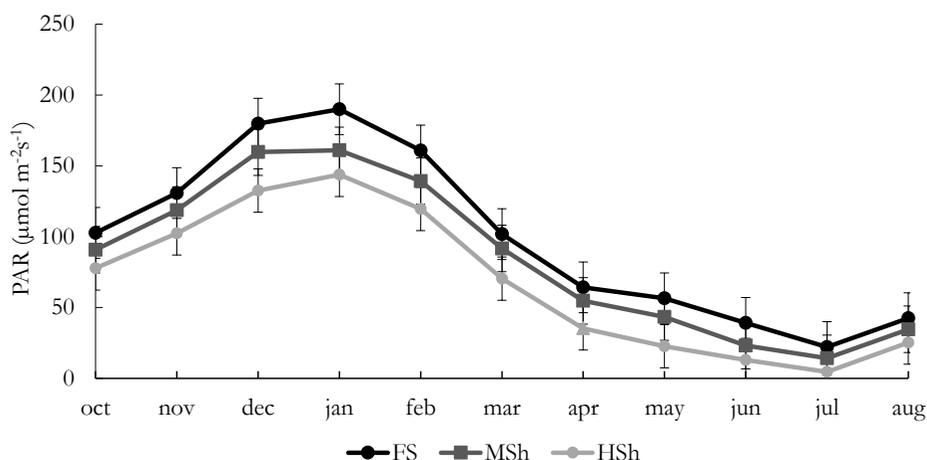


Figure 3. Monthly average photosynthetic active radiation (PAR) (400-700 nm waveband) on the three levels of shading. HSh, Heavy shade; MSh, Medium shade; FS, Full sunlight.

Figura 3. Promedio mensual de radiación fotosintética activa (PAR) (400-700 nm de banda de onda) en los tres niveles de sombreadamiento. FS, luz solar completa; MSh, sombra media; HSh, sombra alta.

Table 2. Transmissivity of the shaded treatments as a percentage of the photosynthetic active radiation (PAR) for sunny days at three different solar angle elevation. Values in parentheses correspond to the daily PAR for full sunlight ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

Cuadro 2. Transmisividad de los tratamientos sombreados como porcentaje de la radiación fotosintéticamente activa (PAR) para los días soleados en tres elevaciones diferentes del ángulo solar. Los valores entre paréntesis corresponden al PAR diario para luz solar completa ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

Solar angle at noon	53.4°	67.4°	45.7°	32.2°
Treatment	Spring	Summer	Autumn	Winter
FS	100% (132.2)	100% (183.0)	100% (64.9)	100% (40)
MSh	68%	83%	78%	88%
HSh	44%	69%	40%	51%

Table 3. The effect of shading on *L. perenne* (Lp) and *A. capillaris* (Ac) dry matter production, tiller density, crude protein, leaf length, leaf area and chlorophyll content index (Mean \pm SE) as part of naturalized pastures.**Cuadro 3.** El efecto del sombreado sobre *L. perenne* (Lp) y *A. capillaris* (Ac) en la producción de materia seca, densidad de macollos, proteína cruda, longitud lamina, área foliar e índice del contenido de clorofila (Media \pm SE) como parte de praderas naturalizadas.

	FP						NFP					
	Lp			Ac			Lp			Ac		
	FS	MSh	HSh	FS	MSh	HSh	FS	MSh	HSh	FS	MSh	HSh
DM (mg)	1.42 \pm 0.20 ^e	1.5 \pm 0.25 ^b	1.63 \pm 0.20 ^a	1.34 \pm 0.18 ^a	1.21 \pm 0.09 ^e	1.25 \pm 0.14 ^b	1.18 \pm 0.3 ^e	1.42 \pm 0.26 ^a	1.27 \pm 0.22 ^b	1.56 \pm 0.13 ^a	1.39 \pm 0.1 ^c	1.49 \pm 0.18 ^b
TD (tiller m ⁻²)	9493 \pm 668 ^e	15184 \pm 1545 ^a	11001 \pm 345 ^b	9395 \pm 1687 ^a	4761 \pm 295 ^e	6087 \pm 247 ^b	5065 \pm 312 ^e	10602 \pm 1087 ^a	6357 \pm 159 ^b	11518 \pm 1802 ^a	6452 \pm 894 ^e	9910 \pm 1129 ^b
CP (%)	28 \pm 1.9 ^e	33.73 \pm 2.21 ^b	37.89 \pm 6.84 ^a	26.63 \pm 1.87 ^e	30.7 \pm 2.68 ^b	36.23 \pm 1.92 ^a	26.1 \pm 3.34 ^b	26.7 \pm 3.70 ^b	30 \pm 4.13 ^a	20.1 \pm 2.68 ^e	22.6 \pm 2.68 ^b	25.5 \pm 1.92 ^a
LL (cm)	7.87 \pm 0.27 ^e	8.5 \pm 0.28 ^b	9.63 \pm 0.31 ^a	6.5 \pm 0.30 ^b	6.81 \pm 0.34 ^b	7.2 \pm 0.21 ^a	8.25 \pm 0.18 ^a	7.6 \pm 0.26 ^b	7.34 \pm 0.7 ^b	5.7 \pm 0.15 ^b	6.42 \pm 0.23 ^a	6.23 \pm 0.21 ^a
LA (cm ²)	0.41 \pm 0.02 ^b	0.46 \pm 0.02 ^b	0.7 \pm 0.03 ^a	0.35 \pm 0.05 ^e	0.37 \pm 0.03 ^a	0.36 \pm 0.08 ^b	0.45 \pm 0.02 ^a	0.43 \pm 0.03 ^b	0.4 \pm 0.08 ^e	0.16 \pm 0.02 ^e	0.29 \pm 0.04 ^a	0.27 \pm 0.07 ^b
CCI	15.17 \pm 1.33 ^e	16.4 \pm 0.61 ^b	17.4 \pm 2.34 ^a	16.3 \pm 1.2	16 \pm 0.5	16.4 \pm 1.6	12.4 \pm 1.3 ^b	12.63 \pm 0.5 ^b	14.5 \pm 0.8 ^a	11.4 \pm 0.9 ^b	13.7 \pm 1.3 ^a	13.83 \pm 1.3 ^a

DM, Dry matter production; TD, tiller density; CP, crude protein; LL, leaf length; LA, leaf area; CCI, chlorophyll content index

NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture; HSh, Heavy shade; MSh, Medium shade; FS, Full sunlight. Different letters in each row for the same specie indicate significant differences (* P \leq 0.05; ** P \leq 0.01; *** P \leq 0.001; ns P \geq 0.05).

Lp (Table 5 and Table 6). The increase in soil nutrients level found in FP was in soil mineral nitrogen, Olsen P, total exchangeable bases and CEC with 2.92, 1.21, 2.72 and 12.35 times higher, respectively, than in NFP. While in AlSat and exchangeable Al³⁺ were 7.8 and 4.3 times lower, respectively, than in NFP.

The pasture species responses to increasing shading and to fertilizer addition were different for CP, LL, LA and CCI (Figure 4). FP-Lp had the highest CP in MSh compare to what was obtained in FS and HSh (Figure 4a). Additionally, the CP levels of NFP-Ac decreased in FS

while in MSh and HSh increased. The largest LL was provided by FPLp under HSh (FP-Lp 9.63 cm) (Figure 4b) and the shortest LL was obtained for NFPAc (5.7 cm) under FS (Figure 4b). Lp and Ac responded different under shade treatments for LA (Figure 3c) and CCI (Figure 4d). Particularly, while FP-Ac had similar CCI across shade treatments (Table 5), Lp had higher LA and CCI values than Ac under HSh in both naturalized pastures (Table 5 and Figures 4c, d).

The regression analysis revealed a positive relationship between dry matter production under shading

Table 4. Statistical significance of F ratios from the analysis of variance for *L. perenne* (Lp) and *A. capillaris* (Ac) dry matter production, tiller density, crude protein, leaf length, leaf area and chlorophyll content index (Mean ± SE) as part of naturalized pastures.

Cuadro 4 Significancia estadística de F ratio del análisis de varianza para *L. perenne* (Lp) y *A. capillaris* (Ac) en la producción de materia seca, densidad de macollos, proteína cruda, longitud de lámina, área foliar e índice del contenido de clorofila (Media ± SE) como parte de praderas naturalizadas.

	FP (A)					NFP (A)			
	Shade (B)	Sp (C)	AxB	BxC	AxBxC	Shade (B)	Sp (C)	BxC	AxBxC
	F: 3.21	F: 3.34	F: 2.67	F: 2.84	F: 3.26	F:3.21	F:3.34	F:2.84	F:3.26
DM (mg)	4.435**	12.050**	2.853*	4.701**	8.321**	3.542*	4.022*	3.87*	6.005**
TD (tiller m ⁻²)	3.563*	4.275*	3.457*	4.367**	7.560**	3.420*	2.931ns	1.902ns	2.102ns
CP (%)	4.893**	5.782**	4.801*	3.216*	4.814*	4.006*	4.140*	3.451*	4.315**
LL (cm)	4.375**	3.912*	3.267**	3.876**	7.360**	4.107**	3.509*	3.068*	4.901**
LA (cm ²)	4.502**	3.871*	3.601*	4.620**	9.233**	4.202*	3.012*	3.925**	7.082**
CCI	3.904*	4.551**	4.547*	3.651*	4.078*	3.502*	3.870*	4.028**	3.490*

DM, Dry matter production; TD, tiller density; CP, crude protein; LL, leaf length; LA, leaf area; CCI, chlorophyll content index
 NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture; HSh, Heavy shade; MSh, Medium shade; FS, Full sunlight. Different letters in each row for the same specie indicate significant differences (* P≤ 0.05, ** P≤ 0.01; *** P≤ 0.001; ns P≥ 0.05)

Table 5. Effect of fertilizer addition on Lp and Ac (across shading treatments) dry matter production, tiller density, crude protein, leaf length, leaf area and chlorophyll content index (Mean ± SE) as part of naturalized pastures.

Cuadro 5. Efecto de la adición de fertilizante sobre Lp y Ac (a través de los tratamientos de sombreado), materia seca, densidad de macollos, proteína cruda, longitud de lámina e índice del contenido de clorofila (Media ± SE) como parte de praderas naturalizadas.

	FP		NFP	
	Lp	Ac	Lp	Ac
DM (mg)	1.51±0.1 ^a	1.26±0.14 ^b	1.29±0.2 ^b	1.48±0.14 ^a
TD (tiller m ⁻²)	9849±845 ^a	6522±671 ^b	6626±445 ^b	8842±931 ^a
CP (%)	33.2±3.65 ^a	31.18±2.15 ^b	27.6±3.72 ^a	22.7±2.42 ^b
LL (cm)	8.6±0.28 ^a	6.83±0.28 ^b	7.73±0.38 ^a	6.12±0.19 ^b
LA (cm ²)	0.52±0.02 ^a	0.36±0.05 ^b	0.42±0.04 ^a	0.24±0.04 ^b
CCI	16.3±1.42 ^a	16.2±1.1 ^b	13.1±0.86 ^a	12.9±1.16 ^b

DM, Dry matter production; TD, tiller density; CP, crude protein; LL, leaf length; LA, leaf area; CCI, chlorophyll content index. NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture. Different letters in each row for the same pasture indicate significant differences (* P≤ 0.05, ** P≤ 0.01; *** P≤ 0.001; ns P≥ 0.05)

with TD (MSh: $R^2=0.8$, $P\leq 0.05$; HSh: $R^2=0.7$, $P\leq 0.05$) and LL (MSh: $R^2=0.7$, $P\leq 0.05$, HRh: $R^2=0.6$, $P\leq 0.05$).

The seasonal behavior of both species in FS showed that Fp-Lp had the highest DM, tillering, CP, LL and LA compare with Ac (Table 6) during spring. Fp-Ac had higher CCI than Lp during summer and autumn. In NFP, Ac had the highest DM (autumn and winter) and tillering (summer). The CP levels of NFP-Lp increases in spring and summer over Ac. NFP-Lp had higher LA values than Ac, while there is not differences on CCI during autumn and winter between both species in NFP (Table 6).

DISCUSSION

There is ample knowledge that shading reduces plant yield (Devkota *et al.*, 2009; Li *et al.*, 2014; Ehret *et al.*, 2015). The light regime of the pastures, characterized by periods of full sunlight and severe shading, was accurately simulated using shading structures according to the mean of daily PAR. Previous studies showed that some forage grasses and legumes grown at 50% full sunlight achieved similar dry matter yields to the non-shaded control (Lin *et al.*, 1999). The reduction

under shading by grass species was reported by Moot *et al.* (2007) with *D. glomerata* DM growth rate decreased by 13% under slat shade in the open sunlight, 22% under tree shade and 48% under the trees + slats shade. Similarly, Hawke and Knowles (1997) reported that DM production of *L. perenne* with *T. repens* in silvopastoral systems under tree shade was only 25% of the open sunlight pasture production. In this study, the structure modified the light available for the species. Specifically, the daily PAR for a day in summer was $183 \mu\text{mol m}^{-2} \text{s}^{-1}$ (100% transmissivity) and it was reduced in MSh and HSh. The minimum daily PAR measured at ground level was $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in HSh on July which meant that during winter season there was a tillering rate reduction of 12% with respect to autumn and 18.1% compared to spring (Flores, 2015).

The reduction of light intensity did not reduced tillering population density. *Lolium perenne* tiller number increased under shading, especially under MSh and also under FP conditions more than in NFP. *Agrostis capillaris* tiller number increased in FS more than under MSh and HSh, especially in NFP, where *A. capillaris* was able to express its growth ability under low fertility conditions (Flores *et al.*, 2017). Kyriazopoulos *et*

Table 6. Seasonal behavior of Lp and Ac (under FS) on dry matter production, tiller density, crude protein, leaf length, leaf area and chlorophyll content index (Mean \pm SE) as part of naturalized pastures.

Cuadro 6. Comportamiento estacional de Lp y Ac (sin sombreadamiento) materia seca, densidad de macollos, proteína cruda, longitud de lámina e índice del contenido de clorofila (Media \pm SE) como parte de praderas naturalizadas.

		FP				NFP			
		Spring	Summer	Autum	Winter	Spring	Summer	Autum	Winter
DM (mg)	Lp	1.62 \pm 0.15 ^a	1.27 \pm 1.22 ^a	1.45 \pm 0.3 ^a	1.35 \pm 0.3 ^a	1.57 \pm 0.08	0.97 \pm 0.07 ^b	1.3 \pm 0.06 ^b	0.86 \pm 0.06 ^b
	Ac	1.46 \pm 0.12 ^b	1.23 \pm 0.17 ^b	1.38 \pm 0.13 ^b	1.29 \pm 0.2 ^b	1.52 \pm 0.3	1.49 \pm 0.4 ^a	1.58 \pm 0.05 ^a	1.66 \pm 0.3 ^a
	Signif.	**	*	*	*	ns	*	*	*
TD (tiller m ⁻²)	Lp	11759 \pm 519 ^a	7550 \pm 345 ^b	10321 \pm 1017 ^a	8345 \pm 783 ^a	6578 \pm 1046 ^b	4141 \pm 754 ^b	5091 \pm 745 ^b	4451 \pm 931 ^b
	Ac	10998 \pm 804 ^b	10449 \pm 560 ^a	8828 \pm 1002 ^b	7299 \pm 123 ^b	10154 \pm 1036 ^a	12248 \pm 980 ^a	11697 \pm 1143 ^a	11969 \pm 1040 ^a
	Signif.	*	**	**	*	**	***	***	***
CP (%)	Lp	33.03 \pm 3.21 ^a	25.1 \pm 2.40 ^a	29 \pm 3.54 ^a	26.63 \pm 3.47	28.78 \pm 4.2 ^a	26.8 \pm 3.7 ^a	24.85 \pm 2.8 ^a	23.85 \pm 3.1 ^a
	Ac	28.2 \pm 2.57 ^b	23.7 \pm 1.13 ^b	27.9 \pm 2.50 ^b	26.6 \pm 2.31	23.4 \pm 2.8 ^b	20.8 \pm 1.71 ^b	19.75 \pm 3.2 ^b	16.6 \pm 2.4 ^b
	Signif.	*	*	*	ns	*	**	*	**
LL (cm)	Lp	8.7 \pm 0.25 ^a	7.4 \pm 0.6 ^a	8.5 \pm 1.1 ^a	6.9 \pm 0.8	8.7 \pm 0.8 ^a	7.6 \pm 0.4	8.6 \pm 1.4 ^a	8.1 \pm 2.1 ^a
	Ac	7.6 \pm 0.46 ^b	5.9 \pm 1.3 ^b	6.4 \pm 1.03 ^b	6.2 \pm 0.7	4.7 \pm 0.5 ^b	7.1 \pm 0.5	6.8 \pm 0.8 ^b	4.2 \pm 0.7 ^b
	Signif.	*	**	**	ns	**	ns	*	**
LA (cm ²)	Lp	0.7 \pm 0.04 ^a	0.14 \pm 0.06	0.51 \pm 0.07 ^a	0.32 \pm 0.03	0.73 \pm 0.1 ^a	0.16 \pm 0.03 ^b	0.52 \pm 0.08 ^a	0.35 \pm 0.06 ^a
	Ac	0.62 \pm 0.02 ^b	0.13 \pm 0.03	0.36 \pm 0.04 ^b	0.3 \pm 0.08	0.19 \pm 0.07 ^b	0.12 \pm 0.06 ^a	0.17 \pm 0.08 ^b	0.15 \pm 0.05 ^b
	Signif.	*	ns	*	ns	**	*	**	*
CCI	Lp	16.305 \pm 1.1	14.13 \pm 1.4 ^b	15.17 \pm 1.8 ^b	15.03 \pm 0.4	13.5 \pm 0.9 ^a	14.7 \pm 2.1 ^a	12 \pm 1.5	9.3 \pm 0.8
	Ac	16.98 \pm 1.4	15.82 \pm 1.8 ^a	16.9 \pm 1.5 ^a	15.3 \pm 0.7	12.6 \pm 0.7 ^b	11.4 \pm 1.7 ^b	11.8 \pm 1.9	9.585 \pm 1.2
	Signif.	ns	*	*	ns	*	*	ns	ns

DM, Dry matter production; TD, tiller density; CP, crude protein; LL, leaf length; LA, leaf area; CCI, chlorophyll content index. NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture. Different letters in each row for the same pasture indicate significant differences (* $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$; ns $P\geq 0.05$)

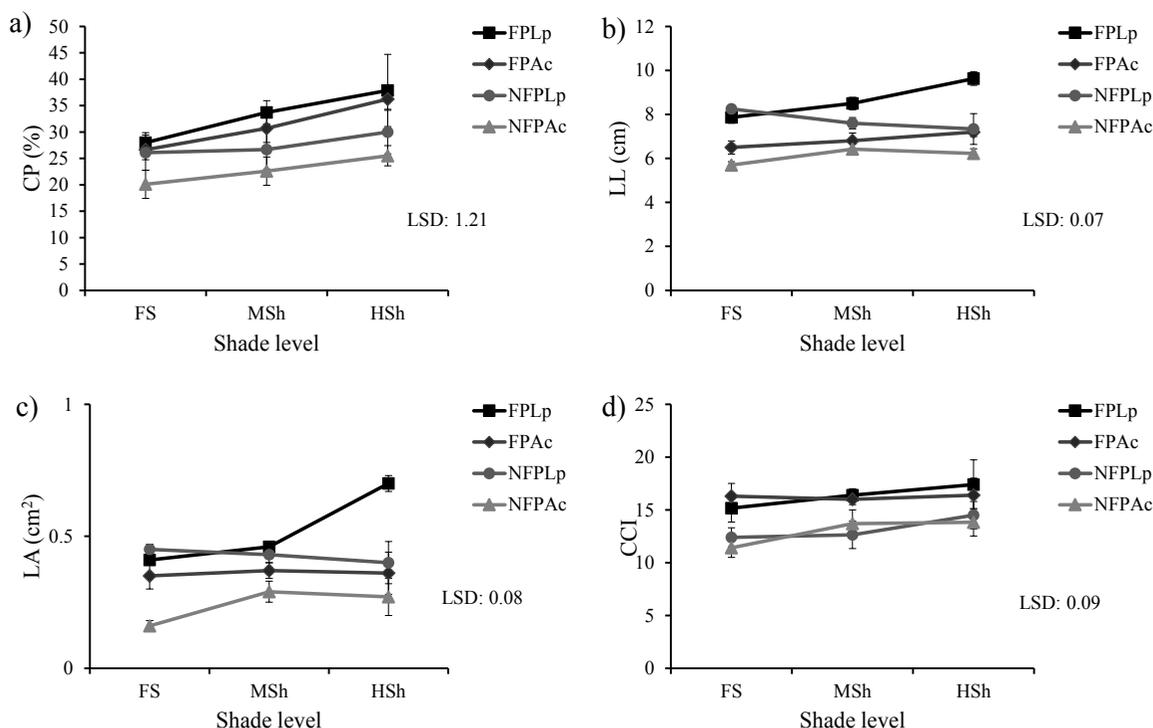


Figure 4. Crude protein (CP) (%), leaf length (LL) (cm), leaf area (LA) (cm²) and chlorophyll content index (CCI) (Mean ± SE) for *Lolium perenne* (Lp) and *Agrostis capillaris* (Ac) under shade treatments. NFP, non fertilized naturalized pasture; FP, naturalized fertilized pasture.

Figura 4. Proteína cruda (CP)(%), largo de lámina (LL), área foliar (LA) (cm²) e índice de contenido de clorofila (CCI) (Media ±SE) para *Lolium perenne* (Lp) y *Agrostis capillaris* (Ac) en sombreado. NFP, pradera naturalizada no fertilizada; FP, pradera naturalizada fertilizada.

al. (2013) also reported that *D. glomerata* had a significant DM increase under shading conditions, not being negatively affected by increasing shading. In contrast, Devkota et al. (2009) evaluating the effect of trees planted to control soil erosion on pasture growth, reported that *L. perenne* tillering was suppressed by tree canopy shading, more strongly than was *Holcus lanatus*, *D. glomerata* and *T. repens*. It was concluded that this response of *L. perenne* under shading made it unsuitable for silvopastoral system use.

The apparent contradiction of the results obtained in this study regarding pasture species growing under shading can be attributable to the conjunction of multiple factors involved in these species development, such the differences in soil nutrients availability and soil water content. This could mean that in temperate pastures, incident light interact with nutrients availability and soil water content where Lp and Ac are more responsive to CP, LL, LA and CCI at higher soil nutrients availability acting both as a shade tolerant plant species. On the contrary, in low nutrients availability both Lp and Ac presented lower levels in CP, LL, LA and CCI. The soil nutrients availability influences positively the lamina length.

The limited soil water availability under drought periods (as in summer season since 21th December to 20th of March) allowed that plants grown under shading were able to grow more than the plants under FS, which was supported by the larger amount of soil moisture under shading (Flores, 2015; Kwon and Woo 2016).

Consistent with these results, were the findings reported by Ordóñez et al. (2018), where *L. perenne* developed a higher concentration of roots in the first 10 cm of soil. The increase of root morphological traits may magnify the water uptake of the pasture and even improve its resilience (Barkaoui et al., 2016). The soil pore system development plays a key role in the root access to available soil water and nutrients (Ordóñez et al., 2018). *Lolium perenne* has a shallow root system, being able to extract less available water from deeper soil horizons compared to other pasture species, such as *Bromus willdenowii* Kunth, *Festuca arundinacea* Schreb. and *Phalaris aquatica* L. (Neal et al., 2012).

In FS, the WC decreased in all seasons with a reduction of 14.3% and 24.5% under MSh and HSh, respectively in FP, while in NFP registered a reduction of 17.6% and 17.2% in MSH and HSh, respectively (Flores, 2015).

In contrast, when WC does not constitute a constraint to plant growth, PAR becomes the larger limiting factor to pasture species herbage mass accumulation (Burner and Belesky 2008; Wang *et al.*, 2016). Several studies have reported that a close relationship between pasture height and tiller population, such that, low tillering rate is consequence of tall pastures and high tillering rate is related to short pastures (Matthew *et al.*, 1995; Sbrissia and Da Silva, 2008). These results have been explained as a consequences of light competition between pasture species, as described by Sackville-Hamilton *et al.* (1995), suggesting that the reduction of light reaching the base of a pasture is one of the main factors that negatively interferes on tillering. Perhaps the shading structure in this study, created an environmental condition with enough WC, N and PAR to favor tiller development especially in MSh, that had the highest tiller development found in this study in FPLp with 18624 tiller m⁻² during autumn with a PAR of 26.1 μmol m⁻² s⁻¹ average.

The increase in CP as PAR declined could be attributed to an increase of foliar N associated to the increment of soil moisture content in shaded conditions (Reynolds, 1995; Peri *et al.*, 2007). It is important to take into account that under shaded conditions, as in the present study, there was probably an increase in soil organic mineralization providing a larger soil N available to be used by the plants (Whitehead, 1995; Table 5; Figure 3a). The higher CCI under shade conditions is explained because FS chlorophyll was more exposed to photooxidative stress that reduced the concentration of the pigment inside the leaf (Triantaphyllides *et al.*, 2008).

It is possible that the larger growth shown by *L. perenne* in MSh resulted from the increase in WC and soil nutrient content under MSh, allowing *L. perenne* to better express its competitive ability prejudicing *A. capillaris* development. The competitive ability showed by Lp could be also due to shade reduced transpiration demands, particularly in dry conditions, maintaining moisture in air and soil (Li *et al.*, 2014).

In other hand, when water was non limiting (late autumn) and temperature was 8 °C average, there was not significant differences in WC between treatments. Nevertheless, the fact that in FP, Lp registered a maximum 17212 tiller m⁻² under MSh, and 9345 tiller m⁻² in FS, showed a significant effect of MSh over FS. This may indicate that the lower tiller density in FS during late autumn was limited mainly by PAR (Flores, 2015).

Leaf morphology modification is an expression of phenotypic plasticity, allowing individual plants to vary their shape, within certain limits, to shading conditions (Ehret *et al.*, 2015). Pasture leaf size and area are function of leaf length (Sbrissia *et al.*, 2010) and tiller density, which allows flexibility to plants, such that they can adjust their morphology under changing defo-

liation conditions when pastures are grazed (Matthew *et al.*, 2000). Changes in the quantity of incident light, can modify stems elongation, promoting tillering (Peri *et al.*, 2007). At lower light intensities, plants with high leaf area tended to have higher photosynthetic rate per unit of nitrogen (Kwon and Woo, 2016).

As happened in the present study, the increase in CP content under shading conditions also has been reported for different pasture species such as *Festuca arundinacea* Schreb (Burner and Brauer, 2003), *Festuca ovina*, *D. glomerata*, *T. repens* and *Trifolium pratense* L. (Parissi and Koukoura, 2009) and *Taraxacum officinale* (Sánchez-Jardón *et al.*, 2010). The longer leaves, larger leaf area, greater CCI found under HSh allowed *L. perenne* and *A. capillaris* to maximize light capture and provide evidence of their shade tolerance, especially when soil nutrients are not limiting plant growth. The relationship between the dry matter production and leaf growth of *L. perenne* and *A. capillaris* under different light incident conditions suggest that species shade tolerance can play a central role in the future management of the pastures. Inside each shade structure there was a change of several microclimatic parameters, of which plant incident light diminishment was the most prominent. During drought periods soil moisture content was higher under shade structures and probably reduced evapotranspiration in periods of full sunlight. In this study Lp was found to be more efficient when the incident light was reduced, displaying a remarkable capacity of acclimation by the change of its morphology during the experimental period. Others studies have provided further evidence of greater enhancement of growth and photosynthesis under shade trees in shade-tolerant species (Valladares and Niinemets, 2008).

The results obtain in this study suggest that shade tolerance of *L. perenne* and *A. capillaris* could be a key to predict the response of plants on temperate pastures are facing with the actual global change such as elevated temperatures, aggressive drought periods or nutrient soil availability .

CONCLUSION

Dry matter accumulation and tiller density of *L. perenne* were stimulated under shading conditions, especially in MSh over *A. capillaris*. The reduction of PAR increased crude protein content, chlorophyll content index, leaf area and leaf length of *L. perenne* and *A. capillaris*, being highest in FP conditions and under HSh. *L. perenne* performs best in shaded areas that are constantly moist. MSh and HSh created an environmental condition with enough WC, N and PAR to favor pasture production, a fact that implies the mitigation of the drought stress effects in the future climate change scenario.

ACKNOWLEDGEMENTS

This study was sponsored by Fund for the Scientific and Technological Development (Fondecyt), Chile, Project No. 1100957. The principal author thanks to the Chilean National Council of Science and Technology (Conicyt) Doctoral Fellowship and Postgraduate Studies Direction of Universidad Austral de Chile.

REFERENCES

- Abraham, E.M., Kyriazopoulos, A.P., Parissi, Z.M., Kostopoulou, P., Karatassiou, M., Anjalanidou, K., Katsouta, C., 2014. Growth, dry matter production, phenotypic plasticity, and nutritive value of three natural populations of *Dactylis glomerata* L. under various shading treatments. *Agroforestry systems* 88(2), 287-299. <https://link.springer.com/article/10.1007/s10457-014-9682-9>
- Anderson, G.W., Moore, R.W., 1987. Productivity in the first seven years of a *Pinus radiata*-annual pasture agroforest in Western Australia. *Australian Journal of Experimental Agriculture* 27(2), 231-238. <http://www.publish.csiro.au/an/EA9870231>
- Bahmani, I., Thom, E.R., Matthew, C., Hooper, R.J., Lemaire, G., 2003. Tiller dynamics of perennial ryegrass cultivars derived from different New Zealand ecotypes: effects of cultivar, season, nitrogen fertiliser, and irrigation. *Australian Journal of Agricultural Research* 54(8), 803-817. <http://www.publish.csiro.au/cp/AR02135>
- Barkaoui, K., Roumet, C., Volaire, F., 2016. Mean root trait more than root trait diversity determines drought resilience in native and cultivated Mediterranean grass mixtures. *Agriculture Ecosystem and Environment* 231, 122-132. <https://www.sciencedirect.com/science/article/pii/S0167880916303498>
- Benkov, M.A., Yatsenko, A.M., Tikhonov, A. N., 2018. Light acclimation of shade-tolerant and sun-resistant *Tradescantia* species: photochemical activity of PSII and its sensitivity to heat treatment. *Photosynthesis research* 1-12. <https://link.springer.com/article/10.1007/s11120-018-0535-7>
- Bremner, J.M., 1996. Nitrogen-Total, in: Sparks, D.L. (Ed.), *Methods of Soil Analysis. Part 3-Chemical Methods*. SSSA, Inc., Madison, WI, USA, pp. 1085-1121.
- Burner, D.M., Belesky, D.P., 2008. Relative effects of irrigation and intense shade on productivity of alley-cropped tall fescue herbage. *Agroforestry Systems* 73(2), 127-139. <https://link.springer.com/article/10.1007/s10457-008-9118-5>
- Burner, D.M., Brauer, D.K., 2003. Herbage response to spacing of loblolly pine trees in a minimal management silvopasture in southeastern USA. *Agroforestry Systems* 57(1), 69-77. <https://link.springer.com/article/10.1023/A:1022943224478>
- Byrt, C.S., 2011. C4 plants as biofuel feedstocks: optimising biomass production and feedstock quality from a lignocellulosic perspective. *Journal of Integrated Plant Biology* 53(2), 120-135. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1744-7909.2010.01023.x>
- Cerovic, Z.G., Masdoumier, G., Ghozlen, N.B., Latouche, G., 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia plantarum* 146(3), 251-260. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-3054.2012.01639.x>
- Chory, J., 2010. Light signal transduction: an infinite spectrum of possibilities. *The Plant Journal* 61(6), 982-991. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-313X.2009.04105.x>
- Centro de Información de Recursos Naturales (CIREN), 2003. *Estudio Agrológico X región. Descripción de Suelos Materiales y Símbolos*. Publicación CIREN N° 123, Santiago, Chile.
- da Silveira Pontes, L., Louault, F., Carrère, P., Maire, V., Andueza, D., Soussana, J.F., 2010. The role of plant traits and their plasticity in the response of pasture grasses to nutrients and cutting frequency. *Annals of botany* 105(6), 957-965. <https://academic.oup.com/aob/article/105/6/957/94275>
- Dec, D., Dörner, J., Balocchi, O., López, I., 2012. Temporal dynamics of hydraulic and mechanical properties of an Andosol under grazing. *Soil and Tillage Research* 125, 44-51. <https://www.sciencedirect.com/science/article/pii/S0167198712001225>
- Devkota, N.R., Kemp, P.D., Hodgson, J., Valentine, I., Jaya, I.K.D., 2009. Relationship between tree canopy height and the production of pasture species in a silvopastoral system based on alder trees. *Agroforestry Systems* 76(2), 363-374. <https://link.springer.com/article/10.1007/s10457-008-9192-8>
- Dingkuhn, M., Luquet, D., Quilot, B., de Reffye, P., 2005. Environmental and genetic control of morphogenesis in crops: towards models simulating phenotypic plasticity. *Crop and Pasture Science* 56(11), 1289-1302. <http://www.publish.csiro.au/CP/AR05063>
- Dodd, M.B., McGowan, A.W., Power, I.L., Thorrold, B.S., 2005. Effects of variation in shade level, shade duration and light quality on perennial pastures. *New Zealand Journal of Agricultural Research* 48, 531-543. <https://doi.org/10.1080/00288233.2005.9513686>
- Ehret, M., Graß, R., Wachendorf, M., 2015. The effect of shade and shade material on white clover/perennial ryegrass mixtures for temperate agroforestry systems. *Agroforestry systems* 89(3), 557-570. <https://link.springer.com/article/10.1007/s10457-015-9791-0>
- Erickson, B., Nosanchuk, T., 1992. *Understanding data*. McGraw-Hill Education Maidenhead, Berkshire, UK.
- Flores, P., 2015. *Interacciones ecosistémicas y cambios en las relaciones prateras al mejorar praderas naturalizadas degradadas con pastoreo ovino intensivo*. Tesis Doctorado en Ciencias Agrarias, Universidad Austral de Chile. 214 p. <http://cybertesis.uach.cl/tesis/uach/2015/egf634i/doc/egf634i.pdf>
- Flores, P.G., López, I.F., Kemp, P.D., Dörner, J., Zhang, B., 2017. Prediction by decision tree modelling of the relative magnitude of functional group abundance in a pasture ecosystem in the south of Chile. *Agriculture, Ecosystems & Environment* 239, 38-50. <https://www.sciencedirect.com/science/article/pii/S0167880917300300>
- Gianoli, E., González-Teuber, M., 2005. Environmental heterogeneity and population differentiation in plasticity to

- drought in *Convolvulus chilensis* (Convolvulaceae). *Evolutionary Ecology* 19(6), 603-613. <https://link.springer.com/article/10.1007/s10682-005-2220-5>
- González-Reyes, A., Muñoz, A., 2013. Cambios en la precipitación de la ciudad de Valdivia (Chile) durante los últimos 150 años. *Bosque (Valdivia)* 34(2), 191-200. https://scielo.conicyt.cl/scielo.php?pid=S0717-92002013000200008&script=sci_arttext&tlng=en
- Hawke, M.F., Knowles, R.L., 1997. Temperate agroforestry in New Zealand, in: Gordon A.M., Newman S.M. (Eds.), *Temperate Agroforestry Systems*. CAB International, UK, Wallingford.
- Instituto Nacional de Investigación de Recursos Naturales-Universidad Austral de Chile (IREN-UACH), 1978. Estudio de los suelos de la Provincia de Valdivia. (accessed 04.06.16) <http://bibliotecadigital.ciren.cl/handle/123456789/22892>
- Kami, C., Lorrain, S., Hornitschek, P., Fankhauser, C., 2010. Lightregulated plant growth and development. *Current Topics in Developmental Biology* 91, 29-66. <https://www.sciencedirect.com/science/article/pii/S0070215310910028>
- Kwon, M.Y., Woo, S.Y., 2016. Plants' responses to drought and shade environments. *African Journal of Biotechnology* 15(2), 29-31. <https://www.ajol.info/index.php/ajb/article/view/132725>
- Kyriazopoulus, A.P., Koukoura, Z., Nastis, A.S., 1999. Effects of wild cherry (*Prunus avium*) and grazing on the herbaceous understorey and its crude protein content. *Grassland Science in Europe*, 4, 187-190.
- Kyriazopoulos, A.P., Abraham, E.M., Parissi, Z.M., Koukoura, Z., Nastis, A.S., 2013. Forage production and nutritive value of *Dactylis glomerata* and *Trifolium subterraneum* mixtures under different shading treatments. *Grass and Forage Science*, 68(1), 72-82. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2494.2012.00870.x>
- Li, J., Terzaghi, W., Deng, X.W., 2012. Genomic basis for light control of plant development. *Protein and Cell* 3(2), 106-116. <https://link.springer.com/article/10.1007/s13238-012-2016-7>
- Li T., Liu L.-N., Jiang C.-D., Liu Y.-J., Shi L., 2014. Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. *Journal of Photochemistry and Photobiology Biology* 137, 31-38.
- Lichtenthaler, H.K., Babani, F., Navrátil, M., Buschmann, C., 2013. Chlorophyll fluorescence kinetics, photosynthetic activity, and pigment composition of blue-shade and half-shade leaves as compared to sun and shade leaves of different trees. *Photosynthesis Research* 117(1-3), 355-366. <https://link.springer.com/article/10.1007/s11120-013-9834-1>
- Lin, C.H., McGraw, R.L., George, M.F., Garrett, H.E., 1999. Shade effects on forage crops with potential in temperate agroforestry practices. *Agroforestry Systems* 44(2-3), 109-119. <https://link.springer.com/article/10.1023/A:1006205116354>
- López, I.F., Kemp, P.D., Dörner, J., Descalzi, C.A., Balocchi, O.A., García, S., 2013. Competitive strategies and growth of neighbouring *Bromus valdivianus* Phil. and *Lolium perenne* L. plants under water restriction. *Journal of Agronomy and Crop Science* 199(2), 449-459. <https://onlinelibrary.wiley.com/doi/abs/10.1111/jac.12032>
- Martínez-García, J.F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemí, M., Bou-Torrent, J., 2010. Regulatory Components of Shade Avoidance Syndrome. *Advances in Botanical Research* 53, 65-116. <https://www.sciencedirect.com/science/article/pii/S0065229610530039>
- Matthew, C., Lemaire, G., Sackville-Hamilton, N.R., Hernández-Garay, A., 1995. A modified self-thinning equation to describe size/density relationships for defoliated swards. *Annals of Botany* 76(6), 579-587. <https://academic.oup.com/aob/article-abstract/76/6/579/207994>
- Matthew, C., Assuero, S.G., Black, C.K., Sackville-Hamilton, N.R., 2000. Tiller dynamics of grazed swards, in: Lemaire, G., Hodgson, J., Moraes, A., Carvalho, P.C.F., Nabinger, C. (Eds.), *Grassland Ecophysiology and Grazing Ecology*. CABI Publishing, Wallingford, pp. 127-150.
- Matthews, P.N.P., Harrington, K.C., Hampton, J.G., 2004. Management of grazing systems in New Zealand, in: White, J., Hodgson, J. (Eds.), *New Zealand Pasture and Crop Science*, Oxford University Press, pp. 153-174.
- Moot, D.J., Matthew, C., Kemp, P.D., Scott, W.R., 2007. Husbandry and role of pastures and forage crops in grazing systems. *Pasture and Supplements for Grazing Animals. Occasional Publication* 14, 23-33.
- Neal, J., Murphy, S., Harden, S., Fulkerson, W., 2012. Differences in soil water content between perennial and annual forages and crops grown under deficit irrigation induced by the dairy industry. *Field Crops Research* 137, 148-162. <https://www.sciencedirect.com/science/article/pii/S0378429012002390>
- Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circ. 939. U.S. Gov. Print. Office, Washington. D.C. <https://archive.org/details/estimationofavai939olse>
- Ordóñez, I., López, I.F., Kemp, P.D., Descalzi, C.A., Horn, R., Zúñiga, F., Dörner, J., 2018. Effect of pasture improvement managements on physical properties and water content dynamics of a volcanic ash soil in southern Chile. *Soil and Tillage Research* 178, 55-64. <https://www.sciencedirect.com/science/article/pii/S0167198717302143>
- Parissi, Z.M., Koukoura, Z., 2009. Effect of fertilization and artificial shading on N and various mineral content of herbaceous species. *Options Méditerranéennes* 85, 159-164. <http://om.iamm.fr/om/pdf/a85/00801001.pdf>
- Page, A.L., Miller, R.H., Keeney, D.R., 1982. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, second ed. American Society of Agronomy and Soil Science Society of America Publisher, Wisconsin.
- Peri, P.L., Lucas, R.J., Moot, D.J., 2007. Dry matter production, morphology and nutritive value of *Dactylis glomerata* growing under different light regimes. *Agroforestry Systems* 70(1), 63-79. <https://link.springer.com/article/10.1007/s10457-007-9029-x>
- Radcliffe, J.E., 1974. Seasonal distribution of pasture production in New Zealand: II. Southland Plains. *New Zealand Journal of Experimental Agriculture* 2(4), 341-348. <https://doi.org/10.1080/03015521.1974.10427693>
- Reynolds, S.G., 1995. *Pasture-Cattle-Coconut Systems*. FAO, RAPA (Regional Office for Asia and the Pacific), Bangkok. <https://www.cabdirect.org/cabdirect/abs->

- tract/19960611793
- Sackville-Hamilton, N.R., Matthew, C., Lemaire, G., 1995. In defence of the $-3/2$ boundary rule: a re-evaluation of self thinning concepts and status. *Annals of Botany* 76, 569-577.
- Sadzawka, A., Carrasco, M., Grez, R., Mora, M., Flores, H., Neaman, A., 2006. Métodos de análisis recomendados para los suelos de Chile. *Serie Actas N° 34*. Instituto de Investigaciones Agropecuarias, Santiago.
- Sánchez-Jardón, L., Acosta, B., Del Pozo, A., Casado, M.A., Ovalle, C., Elizalde, H.F., De Miguel, J.M., 2010. Grassland productivity and diversity on a tree cover gradient in *Nothofagus pumilio* in NW Patagonia. *Agriculture, Ecosystems & Environment*, 137(1-2), 213-218. <https://doi.org/10.1016/j.agee.2010.02.006>
- Sbrissia, A.F., Da Silva, S.C., 2008. Compensação tamanho/densidade populacional de perfilhos em pastos de capimmarandu. *Revista Brasileira de Zootecnia* 37(1), 35-47. <http://www.scielo.br/pdf/rbz/v37n1/v37n1a05>
- Sbrissia, A.F., Da Silva, S.C., Sarmiento, D.O., Molan, L.K., Andrade, F.M., Gonçalves, A.C., Lupinacci, A.V., 2010. Tillering dynamics in palisadegrass swards continuously stocked by cattle. *Plant Ecology* 206(2), 349-359. <https://link.springer.com/article/10.1007/s11258-009-9647-7>
- Schöttler, M.A., Tóth, S.Z., 2014. Photosynthetic complex stoichiometry dynamics in higher plants: environmental acclimation and photosynthetic flux control. *Frontiers in Plant Science* 5, 188. <https://doi.org/10.3389/fpls.2014.00188>
- Steel, R.G., Torrie, J.H., Dickey, D.A., 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, third ed. McGraw-Hill, New York.
- Tharmaraj, J., Chapman, D.F., Nie, Z.N., Lane, A.P., 2008. Herbage accumulation, botanical composition, and nutritive value of five pasture types for dairy production in southern Australia. *Australian Journal of Agricultural Research* 59(2), 127-138. <http://www.publish.csiro.au/cp/ar07083>
- Triantaphylides, C., Krischke, M., Hoeberichts, F.A., Ksas, B., Gresser, G., Havaux, M., Mueller, M.J., 2008. Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. *Plant Physiology*, 148(2), 960-968. <http://www.plantphysiol.org/content/plantphysiol/148/2/960.full.pdf>
- Valladares, F., Niinemets, Ü., 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics* 39, 237-257. <https://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.39.110707.173506>
- Van Reeuwijk, L.P., 1986. *Procedures for Soil Analysis*, sixth ed. International Soil Reference and Information Centre (ISRIC), Wageningen.
- Wahl, S., Ryser, P., Edwards, P.J., 2001. Phenotypic plasticity of grass root anatomy in response to light intensity and nutrient supply. *Annals of Botany* 88(6), 1071-1078. <https://academic.oup.com/aob/article/88/6/1071/2587296>
- Wang, L., Wang, J., Liu, W., Gan, Y., Wu, Y., 2016. Biomass allocation, compensatory growth and internal C/N balance of *Lolium perenne* in response to defoliation and light treatments. *Polish Journal of Ecology*, 64(4), 485-499. <http://www.bioone.org/doi/abs/10.3161/15052249PJE2016.64.4.004>
- Zhai, T., Mohtar, R.H., Gillespie, A.R., von Kiparski, G.R., Johnson, K.D., Neary, M., 2006. Modeling forage growth in a Midwest USA silvopastoral system. *Agroforestry Systems* 67(3), 243-257. <https://link.springer.com/article/10.1007/s10457-005-3823-0>
- Whitehead, D.C., 1995. *Grassland Nitrogen*. CAB International, Wallingford.
- Zhu, X.G., Long, S.P., Ort, D.R., 2010. Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology* 61, 235-261. <https://www.annualreviews.org/doi/abs/10.1146/annurev-arplant-042809-112206>