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Persistencia de gramíneas forrajeras invernales perennes en sistemas silvopastoriles: dinámica de aparición y mortalidad de macollos bajo árboles de *Eucalyptus spp* y pleno sol

Persistence of winter forage grasses in Silvopastoral Systems: appearance and mortality dynamics of of tillers under *Eucalyptus spp* trees and full sun

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*A*bstract

The present study was carried out with the objective of knowing the dynamics of summer survival of perennial C3 grasses in subtropical environments under a microclimate modified by the tree component. Three species grown in conditions of full sun and silvopastoral systems (SPS) (*Dactylis glomerata, Festuca arundinacea* and *Holcus lanatus*) were evaluated, with the hypothesis that the persistence of C3 summer grasses in SPS depends on the ability of the genotype to adapt to the understory microclimate. Forage species were evaluated under tree treatments: two forests with 20 m wide alleys, oriented North-South (NS), East-West (EW) and full sun. The population density of tillers (tillers.m^{-2}) at the beginning of the summer for *Dactylis, Fescue* and *Holcus* respectively was 1.933, 2.867 and 2.156 in NS; 2.289, 3.044, 2.567 in EW and 3.017, 4.500, 4.667 under full sun. The values for the same variable at the end of the study (March) were 2.633, 1.644 and 22 (NS); 2.056, 944 and 0 (EW); 1.750, 3.317 and 533 under full sun ($P < 0.05$). The results showed that the mortality of Fescue tillers accelerated when the amount of light was reduced. *Holcus* showed higher mortality throughout the summer, with an increase below the forest. *Dactylis* was more persistent below forests, especially in the N-S, showing it could be a promising species for SPS.

Key words: *tillers, Festuca, Dactylis, Holcus, survival.*

Resumen

El presente estudio fue realizado con el objetivo de conocer la dinámica de supervivencia estival de gramíneas C3 perennes en ambientes subtropicales bajo un microclima modificado por el componente arbóreo. Fueron evaluadas tres especies cultivadas en condiciones de pleno sol y sistemas silvopastoriles (SPS) (*Dactylis glomerata*, *Festuca arundinacea* y *Holcus lanatus*), con la hipótesis de que la persistencia de gramíneas C3 de verano en SPS depende de la capacidad del genotipo para adaptarse al microclima

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del sotobosque. Se evaluó las especies forrajeras bajo tres tratamientos, dos bosques con callejones de 20 m de ancho, con orientaciones Norte-Sur (NS) y Este-Oeste (EW) y a pleno sol. La densidad poblacional de macollos (macollos.m^{-2}) al comienzo del verano para Dactylis, Festuca y Holcus respectivamente fue 1.933, 2.867 y 2.156 en NS; 2.289, 3.044 y 2.567 en EW y 3.017, 4.500, 4.667 a pleno sol. Los valores para la misma variable al final del estudio (marzo) fueron 2.633, 1.644 y 22 (NS); 2.056, 944 y 0 (EW); 1.750, 3.317 y 533 a pleno sol (P <0,05). Los resultados mostraron que la mortalidad de macollos de Festuca se aceleró cuando se redujo la cantidad de luz. Holcus mostró mayor mortalidad a lo largo del verano, con incremento debajo del bosque. Dactylis fue más persistente debajo de los bosques, especialmente en el N-S, mostrándose una especie promisoria para SPS.

Palabras clave: *tillers, Festuca, Dactylis, Holcus, survival.*

Introduction

The use of silvopastoral systems (SPS) is considered a climate smart agricultural practice, since it represent a strategy to ecological restoration, C sequestration and conservation of water and biodiversity resources, while ensuring agricultural productivity (Ibrahim *et al*. 2010). These systems intentionally combine livestock with trees or other woody perennials and forages on the same unit of land such that mutual benefits to each component arise (Jose *et al*. 2017). Despite the positive microclimatic changes to the understory crops generated by the tree presence, like a thermal buffer effect (lower soil and air temperatures in summer and higher in winter) and the increase in the humidity in the soil (Karki and Goodman 2015), in some situations the reduction of the quantity and quality of solar radiation can compromise their performance. Thus, the spatial arrangement of the trees is a critical aspect determinant of light transmissivity (Jose *et al*. 2017), which constitutes the main limiting resource for pasture growth in these situations. The apparent movement of the sun continuously alters the spatial and temporal projection of the tree shade on the pasture, which is modified by dasometric characteristics of the trees and the orientations of the rows. At medium latitudes, North–South tree lines achieve an homogeneous irradiance of the crop in the alley, with lower intra-annual variability compared to East-West lines (Dupraz *et al*. 2018).

The forage species used in the understory also needs to be adapted to the microclimate environment of a specific system (Jose *et al*. 2017). Some C3 species with moderate shade can maintain relatively productive, since they present physiological characteristics that allow them to maintain high photosynthetic rates under shade (Sage and McKown 2006). *Festuca arudinacea* (tall fescue) and *Dactylis glomerata* are two of the main perennial species cultivated in agroforestry systems (Peri *et al*. 2002; Mercier *et al*. 2020), being that *Dactylis* has been reported to be more tolerant to shade than other C3 grasses (Peri *et al*. 2002; Mercier *et al*. 2020; Belesky *et al*. 2011). Some experiences in New Zealand and Uruguay also showed the positive tolerance responses of the biannual grass *Holcus lanatus* (Yorkshire fog*)* to shading (Devkota *et al*. 2009; Olmos *et al*. 2011). The general response of the grasses to the reduction in photosynthetic active radiation (PAR) and red:far red ratio (R:FR) under tree canopy include reduced tillering, stem elongation and carbohidrate allocation to shoot over roots (Wherley *et al*. 2005). However, tree canopies can reduce temperature during summer months so that forage heat stress can be alleviated (Jose *et al*. 2017).

In subtropical climates, summers are hot and evapotranspiration generally exceeds rain fall, conditions that can be detrimental for the persistence of cool season grasses (Easton *et al*. 1994). Being that dry summers with high temperatures are expected to be more common in South America due to regional trends of increase in the number of heat waves and droughts (Carril *et al*. 2016), alternatives to improve C3 grasses persistence needs to be implement. Enhancing pasture persistence is crucial to achieve more sustainable grass-based animal production systems (Jáuregui *et al*. 2017), which improves soil cover and its nutrient conservation (Campbell *et al*. 1987) and allows for better primary and secondary productivity, reducing production costs (Parsons *et al*. 2011). Since the tiller survival is one of the main features that enables pasture persistence, the lower temperature and higher humidity (of the soil and the air) prevalent in silvopastoral systems could prevent tiller mortality (and perhaps promote its appearance) during summer. However, the persistence of some C3 grasses depends of a higher tiller density at the end of spring (Jáuregui *et al*. 2017), but tillering is negatively affected by the reduction in PAR and R:FR in silvopastoral systems. In this way, our hypothesis was that summer C3 grasses persistence in SPS is dependent of the genotype capacity to adapt to the understory microclimate generated by trees. The objective of this study was to determine the summer turnover of tillers of *Festuca arundinacea*, *Dactylis glomerata* and *Holcus lanatus* planted under *Eucalyptus spp.* forest with two different row orientations and relate it to the microclimate generated by tree presence.

Materials and Methods

The present study was performed at the municipality of Fraile Muerto, Cerro Largo, Uruguay (32 ° 35'S, 54 ° 15'W). The climate type was described as Cfa according to Köppen's classification (Peel *et al*. 2007) a subtropical humid climate without a defined dry season along the year. The soil texture was clay loam, with percentages of clay, silt and sand of 20.0, 28.0 and 52.0 in open pasture, 28.4, 29.0 and 42.6 North-South forest, and 28.6, 27.6, and 43.8 at

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East-West orientation forests respectively, at a 0-20 cm dept. Soil pH was 4.9 in open pasture and East-West forest, and 4.7 at North-South forest. All treatments were fertilized with nitrogen and phosphorus adjusted according to soil analysis.

Clones of *Eucalyptus grandis* x *E. tereticornis* trees were planted in 2009 at 294 trees.ha^{-1} (3.4m within rows and 10m between rows) in two orientations: North-South (N-S forest) and East-West (E-W forest). Trees were pruned in 2013 and thinned in 2018 by alternating removal of entire tree lines, generating a final density of 147 trees.ha^{-1} (3.4 m within rows and 20 m between rows). At age 11, trees had a mean height of 30 m. The control was carried out under the open sky with full sun conditions. The experimental design used for the pasture experiment at each site was randomized complete blocks with three replications, with the treatments being the different forage species. The dimensions of the plots were 20 m x 3.4 m (68 square meters) in the experiments placed under shade, while the plots under full open sky conditions (control conditions) were 5m x 3m (15 square meters).

The three forage species arranged in random plots, were *Festuca arundinácea* Rizar IGP12, *Dactylis glomerata* INIA LE Oberón and *Holcus lanatus* INIA Virtus sown in autumn 2019. In each plot, 3 fixed frames of 0.1 x 0.1m (area 0.01m^2) were located and all the tillers within the frame, which were counted and identified with cables of the same color. This evaluation began in December 2019 and was repeated once a month until April 2020 (5 dates). In each marking, a different color was used (tiller generations).

PAR accumulated in each condition was monitored with PAR quantum sensors (QSOS-PAR, Apogee Instruments) installed in each forest condition, one in the middle of the alley and the other against the row of trees. Soil and air humidity and temperature sensors were also positioned. All these variables plus rain fall were registered under control conditions.

The variables tiller population density (TPD), percentage of survival of the first generation (TS), tiller death rate (TDR) and tiller appearance rate (TAR) were analyzed using a general combined experiment model (Moore and Dixon 2015) using SAS University Edition software (SAS/STAT®, SAS Institute Inc, NC, USA). Treatment means were compared using Tukey's test (α = 5%).

Results

Microclimatic conditions

The light regime was the microclimatic variable that presented the greatest differences between conditions. Within the presence of trees, the PAR was reduced 19 to 52% compared to what the full sun treatment received (Table 1). However, the shading pattern showed differences between the row orientations and month of measurement. In E-W forest the PAR was greater during December and January than N-S forest, but the situation was the opposite in October and from February to May (Table 1). Rows at E-W forest were parallel to the apparent movement of the sun, and that caused more radiation accumulated in the alley over the end of spring with a maximum amount during summer (when the sun was at its highest apparent height) and decreased considerably when autumn begun. In N-S forest, the sun apparent movement was perpendicular to the alley, which generated a high daily variability of radiation, but with less annual variability in relation to E-W forest (Table 1).

Table 1: Mean and standard error for air temperature (°C), soil temperature (°C), soil water content (m^3/m^3) and accumulated photosynthetically active radiation (PAR, $\mu mol/m^2s$) in three different conditions: without trees (Control (C)) and with tree (*E. Grandis x tereticornis*) rows in two different orientations (North-South (N-S) and East-West (E-W)). Standart error values are presented in parentheses.

Variable	Cond.	Month of evaluation							
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Air temp. (°C)	C	16.5 (0.5)	20.1 (0.4)	21.7 (0.6)	22.8 (0.3)	22.5 (0.6)	22.4 (0.3)	17.3 (0.5)	13.2 (0.6)
	N-S	16.5 (0.5)	19.9 (0.4)	21.6 (0.6)	22.8 (0.3)	22.5 (0.6)	22.4 (0.3)	17.3 (0.5)	13.4 (0.6)
	E-W	16.6 (0.5)	20 (0.4)	21.9 (0.6)	23 (0.3)	22.7 (0.6)	22.3 (0.3)	17.6 (0.5)	14.1 (0.5)
Soil temp. (°C)	C	18.3 (0.4)	23.3 (0.3)	25.8 (0.3)	25.9 (0.2)	26 (0.4)	24.3 (0.3)	19 (0.3)	14.8 (0.3)
	N-S	16.8 (0.4)	20.3 (0.2)	22.1 (0.4)	23.3 (0.2)	23.4 (0.4)	22.7 (0.2)	18.1 (0.3)	14.2 (0.3)
	E-W	16.1 (0.4)	19.5 (0.2)	21.5 (0.4)	22.7 (0.2)	22.2 (0.4)	21.4 (0.2)	17.3 (0.3)	14.1 (0.3)
Soil water content (m^3/m^3)	C	0.35 (<0.01)	0.22 (<0.01)	0.12 (<0.01)	0.07 (<0.01)	0.06 (<0.01)	0.05 (<0.01)	0.08 (<0.01)	0.12 (<0.01)
	N-S	0.26 (<0.01)	0.16 (<0.01)	0.06 (<0.01)	0.04 (<0.01)	0.03 (<0.01)	0.03 (<0.01)	0.04 (<0.01)	0.05 (<0.01)
	E-W	0.22 (<0.01)	0.17 (<0.01)	0.1 (<0.01)	0.08 (<0.01)	0.07 (<0.01)	0.07 (<0.01)	0.08 (<0.01)	0.09 (<0.01)
PAR ($\mu mol/m^2$)	C	30.1 (4.0)	44.4 (4.0)	51.0 (4.0)	48.8 (4.0)	46.7 (4.0)	38.5 (4.0)	25.8 (4.0)	20.6 (4.0)
	N-S	13.4 (2.1)	19.4 (2.1)	23.0 (2.1)	20.7 (2.1)	18.4 (2.1)	14.6 (2.1)	9.2 (2.1)	6.3 (2.1)
	E-W	10.4 (2.8)	19.2 (2.8)	26.5 (2.8)	22.2 (2.8)	16.9 (2.8)	9.7 (2.8)	5.9 (2.8)	4.0 (2.8)

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Table 2: Adjusted means and standard error for tiller population density (TPD, tiller m^{-2}) and tiller survival (TS, %) of *Dactylis glomerata*, *Festuca arundinácea,* and *Holcus lanatus* in three different conditions: without trees (Control) and with tree (*E. Grandis x tereticornis*) rows in two different orientations (North-South (N-S) and East-West (E-W)). Standart error values are presented in parenthesis.

Variable	Condition	Specie	Month of evaluation				
			Dec	Jan	Feb	Mar	Apr
Tiller Population Density (TPD, Tiller m^{-2})	Control	Dactylis	3017 (502)	2317 (502)	2600 (502)	2333 (502)	1750 (502)
		Festuca	4500 (502)	4167 (502)	4517 (502)	3967 (502)	3317 (502)
		Holcus	4667 (502)	2500 (502)	1400 (502)	800 (502)	533 (502)
	E-W	Dactylis	2289 (353)	1833 (353)	2422 (353)	2467 (353)	2056 (353)
		Festuca	3044 (353)	2811 (353)	2900 (353)	2356 (353)	944 (353)
		Holcus	2567 (353)	1322 (342)	511 (342)	22 (342)	0 (342)
	N-S	Dactylis	1933 (299)	1689 (299)	2622 (299)	2944 (299)	2633 (299)
		Festuca	2867 (299)	2489 (299)	2411 (299)	1844 (299)	1644 (299)
		Holcus	2156 (299)	933 (299)	422 (299)	22 (299)	22 (299)
Tiller survival (TS, %)	Control	Dactylis		78.2 (0.06)	51.1 (0.06)	36.5 (0.06)	20.7 (0.06)
		Festuca		92.5 (0.06)	85.8 (0.06)	70.6 (0.06)	52.2 (0.06)
		Holcus		57.3 (0.06)	17.1 (0.06)	5.0 (0.06)	2.4 (0.06)
	E-W	Dactylis		80.5 (0.06)	70.7 (0.06)	62.9 (0.06)	46.2 (0.06)
		Festuca		92.8 (0.06)	88.3 (0.06)	64.7 (0.06)	20.8 (0.06)
		Holcus		52.8 (0.06)	15.4 (0.06)	2.0 (0.06)	0.0 (0.06)
	N-S	Dactylis		88.3 (0.06)	85.0 (0.06)	76.8 (0.06)	63.3 (0.06)
		Festuca		87.8 (0.06)	73.5 (0.06)	52.2 (0.06)	43.4 (0.06)
		Holcus		47.5 (0.06)	12.3 (0.06)	0.0 (0.06)	0.0 (0.06)

The average daily air temperature was similar between the different conditions and ranged from 13.2 to 23 (Table 1). Soil temperature registered an average decrease of 2.1 and 2.8°C under the trees in the N-S and E-W forests; respectively, compared to full sun treatment. Soil moisture showed higher values under full sun during spring, and EW higher than NS, with a tendency to decrease in summer for all three conditions. Thus, during summer, EW condition presented similar soil moisture values than full sun pasture (Table 1).

Tiller population density and tiller survival

In the three conditions, significant differences were detected for the TPD between species, date and their interaction (Table 2).

In all conditions, Holcus in December started with its higher TPD values and ended the period with the lowest values (Table 2). TPD was basically composed of the first generation (Figure 1), especially in the treatments under the forest.

Fescue showed the highest TPD between species under full sun in summer and autumn (Table 2). The number of tillers belonging to the first generation and the contribution of the new generations was essential to reach a higher TPD in autumn (in comparison with the other species). The different performances shown between the forests are mainly due to the low TS in E-W at the beginning of autumn (20.8%), and to the limited contribution of the new generations (Figure 1).

Dactylis began the evaluation period with its highest values of TPD under full sun in comparison to forest treatments and ended the period with its highest values under the trees, especially in the N-S orientation. Under the N-S forest, Dactylis showed a survival rate of 63.3% in april, which was the lowest in the period of study. The tillering and low mortality rates were decisive for the construction of the upper TPD at the end of the summer in N-S (Figure 1). The contribution of the new generations of tillers to the TPD under the forest were far more relevant for Dactylis than for Holcus and Festuca (Figure 1). Dactylis was the specie with the highest survival and tiller density in the forest.

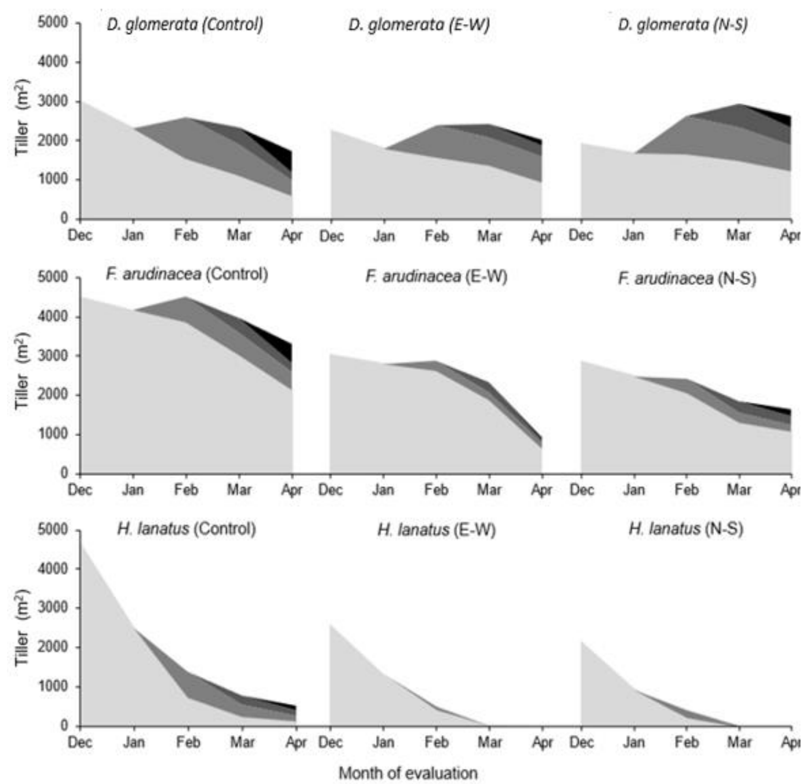

Figure 1. Tiller population density (TPD) of first, second, third and fourth generation (greyscale from lighter to darker respectively) of *Dactylis*, *Festuca arundinácea* and *Holcus lanatus* from January to April in three different conditions: without trees (Control) and with tree (*E. Grandis x tereticornis*) rows in two different orientations (North-South (NS)) and East-West (EW)) (Mean ± SE).

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Appearance and mortality rates

The appearance and mortality rates of tillers presented different behaviors according to the condition and the species (Figure 2). Holcus was the specie with highest mortality in the experiment, showing the maximum values in February and March under trees influence (Figure 2). Fescue showed a high mortality rate under the forest, especially in the E-W orientation in March and April. Mortality rate for Dactylis under full sun was higher than under the forest throughout the period, with maximum differences occurred in February (Figure 2).

Dactylis showed the highest values of appearance rate among species. The higher value was detected during the month of February under the forest in the N-S orientation, but it decreased towards the end of the period, while the values under full sun remain stable throughout the experiment. In the E-W orientation the appearance of tillers was always inferior to the other conditions (Figure 2).

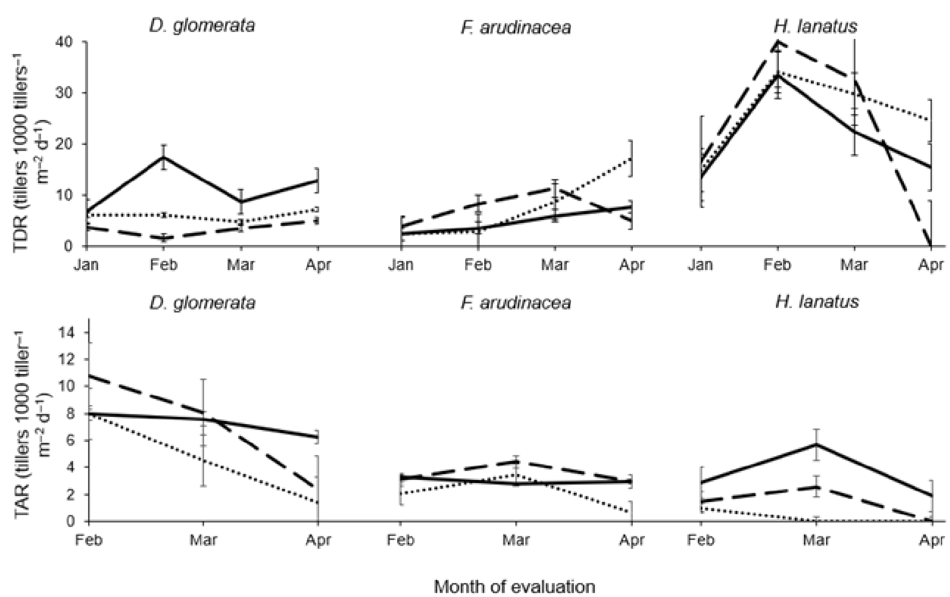

Figure 2. Tiller death rate (TDR) and tiller appearance rate (TAR) for *Dactylis*, *Festuca arundinacea* and *Holcus lanatus* from January to April in three different conditions: without trees (Control (−)) and with tree (*E. Grandis x tereticornis*) rows in two different orientations (North-South (− −) and East-West (····)) (Mean ± SE).

Discussion

The hypothesis that summer C3 grasses persistence in SPS is dependent on the genotype capacity to adapt to the understory microclimate generated by trees was accepted. *Dactylis* was the specie with the best understory persistence in general terms, explained by the highest degree of tiller survival and appearance in relation to the control treatment and the other species, especially when tree lines were N-S oriented. *Holcus lanatus* had a high sensitivity to summer conditions independent of the light regimen, but with all tillers dying under arboreal influence and not in control. Fescue showed better tiller surviving and final tiller density in the open environment, but SPS increased tiller death, which compromised their persistence. The response of each specie was associated to the microclimatic conditions present in each situation.

Despite the great distance between rows of trees (20m), the fact that the plantations were mature (with height of approximately 30 meters), significant PAR reductions were found in the different silvopastoral conditions, sufficient to compromise the tillering of some species. The PAR transmissivity was of 41% in N-S and 38% in E-W in relation to full sun treatment (100% of transmissivity) throughout all the experiment. Despite Dactylis be considered highly tolerant to shade (Peri *et al*. 2002), showed sharp decreases in the tiller population density, photosynthetic rate and dry matter accumulation already with 58% of PAR transmissivity by trees. During the summer, the E-W oriented forest received a greater amount of radiation than the N-S forest, but this relationship was reversed for the rest of the year (spring and autumn). Soil temperature was higher in the full sun treatment throughout all the experiment, and no differences were detected between orientations. Soil moisture was higher under the sun during spring; however, the difference was reduced in summer, getting surpassed by the E-W forest. N-S forest maintained the lowest level of water content in the soil throughout all the experiment. This concur with the results obtained in the study by Karki and Goodman (2015) for mature trees, where the soil temperature was higher in full sun treatment during all the experiment, and soil moisture under the SPS was lower than in open pasture (during December and January). The lower PAR availability in E-W during spring (season that showed high soil humidity and milder temperatures that enables a good cool-season grasses performance) may have generated a priority change in the partition of assimilates to the plants aerial part, compromising the capacity of the tillers to survive when soil moisture is low. Indeed, these tillers are subjected to high intensities of solar radiation and temperatures in summer, aspects that have a negative impact on photosynthetic capacity and tiller surviving (Jáuregui *et al*. 2017).

Dactylis ends the summer period with a higher tiller density than at the beginning of the period. Under N-S forest, Dactylis maintained the highest degree of survival of the first generation in relation to the other treatments and to the other species. In this orientation, Dactylis maintained a low mortality rate of them throughout the period and presented the highest rate of appearance of tillers in February (which was slightly superior in N-S forest than E-W forest). Dactylis appears to keep producing tillers regardless of SPS condition, and subsequent generations can weigh heavily on persistence. Alternatively, Festuca showed less tiller appearance rate under the trees, being this highly related to the limited radiation conditions. References show that in Festuca (Jáuregui *et al*. 2017) the number of tillers present in spring (first generation) was essential for summer persistence and, according to Fernández *et al*. 2004, Festuca under shade has changes in biomass allocation which results in a decrease in carbon allocation to storage organs, thereby reducing the re-growth capacity after herbivory. In the case of *Holcus lanatus*, practically all the tillers present in spring died in summer. Unlike Fescue and Dactylis, Holcus is a biannual specie and its perennialization is determined not only by the survival of tillers (which is generally high during the summer) but also by the production of seeds for the next season and growth. Although the high tiller mortality rate occurred in all situations, some of them were able to survive only in the control treatment, which could help in the persistence of the specie in the following year.

Appearance of tillers rate is better related to full sun regardless of the species. Despite of this, during the month of February, Dactylis showed a high tillers appearance rate under N-S forest, as well as lower tiller mortality, which was manifested in a higher TPD at the end of the period. Duchini *et al*. (2018) showed that for species with functional types classified as conservative growth strategy the persistence is dependent only by the tiller surviving, but those classified as exploitative also depends on the tiller appearance. According to these authors, *Festuca arudinacea* is classified as conservative and *Dactylis* showed a moderately exploitative growth strategy. In the present study, the high tiller density in Fescue at the end of spring (not tiller appearance in summer) contributed to its higher persistence in full sun, which is in accordance to previous works developed in similar climate conditions (Jáuregui *et al*. 2017). Dactylis, on the other hand, achieved a greater degree of adaptation to silvopastoral conditions and performed tiller turnover earlier, which favored its persistence under the trees. The results coincide with the concepts mentioned by Peri *et al*. 2006 and Belesky *et al*. 2011, positioning Dactylis as a species with good performance under moderate shading. In this experiment, the best performance of the species is based on a less mortality through the summer in comparison to a full sun condition.

Conclusions

The results showed that Dactylis was more persistent under the forest, mainly because it presented lower relative mortality rate; and a survival and appearance of tillers rate less susceptible to decreased radiation and soil moisture conditions generated by the forest. The effect was accentuated in

N-S forest due to the higher appearance of tillers during the month of February. The tiller mortality process accelerated when the amount of light was reduced in the case of *Festuca*, while for *Holcus*, the summer conditions caused greater mortality all along, and the process was increased by the presence of the forest. The argumentation of the *Dactylis* performance is based on a lower relationship of tiller mortality to suboptimal light conditions and less relative importance to soil moisture, conditions that were generated under the forest. *Dactylis* appears as a promising species for SPS in terms of summer persistence. This is important, considering that this aspect is key for the longevity of pastures, as well as it is relevant in a context of climate change and adaptation to potential extreme climatic events.

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