

Persistence of winter forage grasses in Silvopastoral Systems: dynamics of appearance and mortality of tillers under Eucalyptus sp. trees and full sunlight

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Research Article

Keywords: *Dactylis glomerata*, *Festuca arundinacea*, *Holcus lanatus*, pasture persistence, tiller survival

Posted Date: February 2nd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3915356/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Agroforestry Systems on December 27th, 2024. See the published version at <https://doi.org/10.1007/s10457-024-01118-z>.

Abstract

The impact of microclimatic changes caused by Silvopastoral Systems (SPS) in subtropical climates on C3 grasses persistence remains unclear, particularly concerning their effects on summer mortality and tiller growth. We hypothesized that the microclimate created by trees with different orientations in SPS would have an impact on the summer C3 grasses' persistence, as measured by the number of tillers per square meter. This study evaluated the survival of two C3 perennial grasses of different tolerance to shade (*Dactylis glomerata*: tolerant, *Festuca arundinacea*: moderately tolerant) and a C3 biannual grass typical of moist environments (*Holcus lanatus*) in full sunlight and SPS with tree rows oriented North-South (NS) and East-West (EW). We observed a decrease in Tiller Population Density (Tiller m⁻²) for *Holcus* in all situations from December to April ($P < 0.05$) (from 2156 to 22 in NS, 2567 to 0 in EW and 4667 to 533 in full sunlight). *Festuca* also decreased in all conditions but showed a relatively better performance (2867 to 1644 in NS, 3044 to 944 in EW and 4500 to 3317 in full sunlight) and *Dactylis* (1933 to 2633 in NS, 2289 to 2056 in EW and 3017 to 1750 in full sunlight). Results showed that *Dactylis* was more persistent under tree canopy, it presented lower mortality rate, especially in N-S oriented tree rows ($P < 0.05$). *Festuca*'s tiller mortality increased under reduced light, while *Holcus* showed higher mortality during summer, exacerbated under tree cover. These findings suggest that *Dactylis* could be a promising species for SPS in subtropical climates, particularly regarding summer persistence.

Introduction

The use of silvopastoral systems (SPS) is considered a climate smart agricultural practice, since it represents a strategy to ecological restoration, C sequestration, and conservation of water and biodiversity resources, while maintaining agricultural productivity (Ibrahim et al. 2010). These systems synergistically integrate livestock, trees or other woody perennials and forages on the same land, yielding mutual benefits (Jose et al. 2017). The presence of trees creates positive microclimatic changes for understory crops, acting as a thermal buffer with lower soil and air temperatures in summer and higher in winter (Karki and Goodman 2015). However, it's important to recognize that in some situations, the diminished quantity and altered quality of solar radiation can compromise the performance of these crops. Therefore, 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 conditions. The apparent movement of the sun continuously alters the spatial and temporal projection of the tree shade on the pasture, which is modified by canopy characteristics of the trees and the orientations of their rows. Notably, at medium latitudes, North-South oriented tree lines provide more uniform crop irradiance in the alleys, with lower intra-annual variability compared to East-West lines (Dupraz et al. 2018). Thus, tree orientation is a relevant aspect when defining the arrangement of a SPS.

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, which can tolerate moderate shade, maintain their productivity as they possess physiological characteristics that allow them to sustain high photosynthetic rates under shaded conditions (Sage and McKown 2006). *Dactylis glomerata*

(orchardgrass; DG) and *Festuca arudinacea* (tall fescue; FA) are two of the main perennial species cultivated in agroforestry systems (Peri et al. 2002; Mercier et al. 2020), with DG reported to be more shade tolerant than FA (Peri et al. 2002; Mercier et al. 2020; Belesky et al. 2011). Studies in New Zealand and Uruguay have also demonstrated the positive responses of the biannual grass *Holcus lanatus* (Yorkshire fog, HL) to shading (Devkota et al. 2009; Olmos et al. 2011), a specie largely present in floodplain wetlands, with relatively low persistence in dry conditions (Boigné et al. 2017). Generally, grasses` response to the reduction in photosynthetic active radiation (PAR) and red: far red ratio (R:FR) under tree canopy include reduced tillering, stem elongation and carbohydrate allocation to shoot over roots (Wherley et al. 2005). Thus, understanding the response of C3 species to incidence of PAR in SPS with different row orientations, is crucial for the stability of the system.

In subtropical climates, summers are hot and evapotranspiration generally exceeds precipitation, conditions that can be detrimental for the persistence of cool season grasses (Easton et al. 1994). Given that dry summers with high temperatures are expected to become more common in South America due to regional trends, including an increase in the frequency of heat waves and droughts (Carril et al. 2016), alternatives to improve C3 grasses persistence needs to be implemented. Enhancing pasture persistence is crucial for achieving more sustainable grass-based animal production systems (Jáuregui et al. 2017), which improve soil cover and nutrient conservation, and allow for better primary and secondary productivity, thereby reducing production costs (Parsons et al. 2011). Since tiller survival is a key factor for pasture persistence, the microclimatic conditions prevalent in SPS could prevent tiller mortality (and perhaps promote its appearance) during summer. However, the persistence of some C3 grasses depends on a higher tiller density at the end of spring (Jáuregui et al. 2017). Therefore, this study hypothesizes that the microclimatic conditions prevalent in SPS may mitigate tiller mortality, and potentially stimulate tiller appearance in grasses better adapted to these conditions, during the challenging summer conditions typical of subtropical climates. This study assesses the summer turnover of tillers of FA, DG and HL planted under *Eucalyptus spp.* SPS with two different row orientations.

Materials and Methods

Location of the experiments

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 throughout the year. However, the rainfall regime has a very significant variation in the amount and distribution among years: the annual precipitation accumulated are between 1200 and 1600 millimeters (mm), with a tendency to register greater water deficiencies in summer and maximum rainfall in winter. The experiment involved growing FA, DG and HL under two tree canopies with 20-m wide alleys, with NS and EW tree rows orientations, and in full sunlight condition. Soil texture was clay loam at 0–20 cm dept, with percentages of clay, silt and sand of 20.0, 28.0 and 52.0 in full sunlight, 28.4, 29.0 and 42.6 in the N-S, and 28.6, 27.6, and 43.8 in

the E-W oriented tree rows; respectively. Soil pH was 4.9 in full sunlight, 4.7 at N-S and E-W oriented tree rows. All treatments were fertilized with nitrogen and phosphorus adjusted according to soil analysis.

Vegetal materials

Clones of *Eucalyptus grandis* x *E. tereticornis* trees were planted in 2009 at 294 trees ha⁻¹ (3.4 m within rows and 10 m between rows) in N-S and E-W orientations. In 2013, trees were pruned, and in 2018, were thinned by alternately removal of entire tree rows, resulting a final density of 147 trees ha⁻¹ (3.4m within rows and 20m between rows). At 11 years post-plantation, trees had a mean height of 30 m. The control was carried out under open sky conditions with full sunlight exposure. The experimental design used for the pasture experiment at each site was randomized complete blocks with three replications, with the treatments being the forage species. The plot dimensions were 20 m x 3.4 m (68 m²) in the experiments placed under shade, while the plots under sunlight conditions were 5 m x 3 m (15 m²). Three forage species, FA Rizar IGP12, DG INIA LE Oberón and HL INIA Virtus were sown in March 2019. The seeding rate was 14, 12 and 7 kg/ha of seeds; respectively.

Evaluation of tiller populations

All species and conditions were evaluated in the first year of study, but during the second year (2020–2021), the evaluations were exclusively centered on the specie with better performance under specific SPS conditions (DG cultivated in N-S forest), which was then compared with its cultivation under full sunlight condition.

In each plot, three fixed frames of 0.1 x 0.1 m were strategically placed – one nearby the alley, one in the middle of the plot, and the other one nearby the tree row. All the tillers within the frame were counted and identified with colored wires of the same color (Hernandez-Garay et al. 1993). These evaluations were performed once a month from December to April, in years 2019–2020 and 2020–2021. On each evaluation, a new color was used to identify the new generation, and previous generations were also counted.

The variable tiller population density (TPD) was estimated by counting the total amount of tillers in each frame for each date expressed in square meters. Tiller survival (TS) was estimated as the percentage of tillers alive in relation to the first set of data (first tiller generation). Tiller death rate (TDR) and tiller appearance rate (TAR) were estimated by the percentage of tillers that died and appear; respectively, for each date in relation to the previous evaluation.

Environmental conditions

Accumulated photosynthetic active radiation 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 other near the tree row. Soil moisture and temperature were measured with sensors located 2 cm below ground surface: four under each tree row orientation and two under full sunlight condition. Air humidity and temperature were measured with sensors located 1 m above ground surface: two in each

tree row orientation and one under full sunlight condition. Precipitation was recorded only under full sunlight condition. All these variables were recorded at 10-minute intervals.

Experimental design and Statistical Analysis

The experiment included three completely randomized block designs (N-S and E-W oriented tree rows and full sunlight condition), therefore, an analysis of combined experiments was performed (Moore and Dixon 2015). The general combined model was performed using a PROC MIXED, using blocks as a random effect, and treatments, species and dates as fixed effects. Adjusted treatment means were compared using Tukey's test ($\alpha = 5\%$). The variables mentioned and climate variables (monthly accumulated PAR radiation, soil moisture and maximum and mean temperature) were evaluated with a Principal Component Analysis (PCA). The software used for analysis of combined experiments and PCA analysis was SAS University Edition software (SAS/STAT®, SAS Institute Inc, NC, USA).

Results

Microclimatic conditions

Overall, the full sunlight condition exhibited higher soil water content throughout the experimental period. Notably, after precipitation events, soil water recharge was more pronounced in full sunlight condition, with a tendency toward homogenization between treatments during periods of lower precipitation. During the spring of 2019 the three treatments demonstrated higher soil relative humidity contents compared to the same season in 2020.

The average daily air temperature remained similar across the different experimental treatments, ranging from 10°C in July 2020 to 24°C in January 2021 (Fig. 2). In contrast, soil temperature was considerably higher in full sunlight during the warmer months, with an average temperature of 26°C during the summer, while in N-S and E-W orientations, were 3°C and 4°C lower; respectively.

PAR reaching the pasture varied according to the month of measurement and the treatment (Fig. 3a and 3b). In the E-W tree orientation, PAR levels were higher in December and January (49% and 55% relative to full sunlight) compared to the N-S tree orientation (46% and 47% relative to full sunlight). However, the E-W tree orientation showed lower PAR values for the rest of the year, with levels around 20% of radiation in the understory. This is in contrast to the N-S tree orientation treatment, where the understory received approximately 30% of PAR.

Tiller population density and tiller survival

For each treatment, a significant interaction was observed among species and date for both TPD and TS during the first year of study (P value < 0.0001) (Tables 1 and 2). Across treatments, HL exhibited elevated TPD values in December, gradually declining to the lowest values at the end of summer. This trend was associated with a decrease in TS, which reached zero at this point. On the other hand, FA and

DG exhibited different dynamics depending on the condition in which they were cultivated. While FA showed a high tiller population density both at the beginning and end of summer under full sunlight conditions, the specie could not maintain the same performance in the forest understory, concluding the summer with a TS of only 20% in the E-W tree row orientation. Conversely, DG displayed an opposite dynamic, demonstrating a high TPD at the end of summer in forest understories, with a TS higher than 60% in the N-S tree row orientation.

Table 1

Adjusted means and standard errors for tiller population density (tillers m^{-2}) of *Dactylis (D.) glomerata*, *Festuca (F.) arundinacea*, and *Holcus (H.) lanatus* for the first year of study in three different treatments: full sunlight and with tree (*E. Grandis x tereticornis*) rows in two different orientations, North-South (N-S) and East-West (E-W).

Specie	Tiller population density (tillers m^{-2})				
	Dec	Jan	Feb	Mar	Apr
<i>Full sunlight</i>					
<i>D. glomerata</i>	3017 (502) ¹ ABC	2317 (502) ABCD	2600 (502) ABCD	2333 (502) ABCD	1750 (502) BCD
<i>F. arundinacea</i>	4500 (502) ^{AB}	4167 (502) ABC	4517 (502) ^{AB}	3967 (502) ABC	3317 (502) ABC
<i>H. lanatus</i>	4667 (502) ^A	2500 (502) ABCD	1400 (502) ^{CD}	800 (502) ^D	533 (502) ^D
<i>E-W</i>					
<i>D. glomerata</i>	2289 (353) ABC	1833 (353) ABCD	2422 (353) ABC	2467 (353) ABC	2056 (353) ABCD
<i>F. arundinacea</i>	3044 (353) ^A	2811 (353) ^A	2900 (353) ^A	2356 (353) ABC	944 (353) ^{BCD}
<i>H. lanatus</i>	2567 (353) ^{AB}	1322 (342) ABCD	511 (342) ^{CD}	22 (342) ^D	0 (342) ^D
<i>N-S</i>					
<i>D. glomerata</i>	1933 (299) ^{AB}	1689 (299) ABC	2622 (299) ^A	2944 (299) ^A	2633 (299) ^A
<i>F. arundinacea</i>	2867 (299) ^A	2489 (299) ^A	2411 (299) ^A	1844 (299) ^{AB}	1644 (299) ABCD
<i>H. lanatus</i>	2156 (299) ^A	933 (299) ^{BCD}	422 (299) ^{CD}	22 (299) ^D	22 (299) ^D

¹The means sharing the same letters within each treatment (full sunlight, N-S, or E-W) do not show significant differences for the interaction species and month of evaluation, adjusted by Tukey test (P < 0.05).

Standard error is presented within parentheses.

Table 2

Adjusted means and standard errors for tiller survival (%) of *Dactylis (D.) glomerata*, *Festuca (F.) arundinacea*, and *Holcus (H.) lanatus* for the first year of study in three different treatments: full sunlight and tree (*E. Grandis x tereticornis*) rows in two different orientations, North-South (N-S) and East-West (E-W).

Specie	Tiller Survival (%)			
	Jan	Feb	Mar	Apr
<i>Full sunlight</i>				
<i>D. glomerata</i>	78.2 (0.06) ^{ABCD}	51.1 (0.06) ^{EF}	36.5 (0.06) ^{FG}	20.7 (0.06) ^{GH}
<i>F. arudinacea</i>	92.5 (0.06) ^{AB}	85.8 (0.06) ^{ABC}	70.6 (0.06) ^{BCDE}	52.2 (0.06) ^{DEF}
<i>H lanatus</i>	57.3 (0.06) ^{CDEF}	17.1 (0.06) ^{GH}	5.0 (0.06) ^H	2.4 (0.06) ^H
<i>E-W</i>				
<i>D. glomerata</i>	80.5 (0.06) ^{ABCD}	70.7 (0.06) ^{BCD}	62.9 (0.06) ^{CDE}	46.2 (0.06) ^{EF}
<i>F. arudinacea</i>	92.8 (0.06) ^{AB}	88.3 (0.06) ^{ABC}	64.7 (0.06) ^{CDE}	20.8 (0.06) ^{FG}
<i>H lanatus</i>	52.8 (0.06) ^{DE}	15.4 (0.06) ^{GH}	2.0 (0.06) ^G	0.0 (0.06) ^G
<i>N-S</i>				
<i>D. glomerata</i>	88.3 (0.06) ^{AB}	85.0 (0.06) ^{AB}	76.8 (0.06) ^{ABC}	63.3 (0.06) ^{BCD}
<i>F. arudinacea</i>	87.8 (0.06) ^{AB}	73.5 (0.06) ^{ABC}	52.2 (0.06) ^{CD}	43.4 (0.06) ^{DE}
<i>H lanatus</i>	47.5 (0.06) ^{CD}	12.3 (0.06) ^{EF}	0.0 (0.06) ^F	0.0 (0.06) ^F

¹The means sharing the same letters within each condition (Full sunlight, N-S, or E-W) do not show significant differences for the interaction species and month of evaluation, adjusted by Tukey test (P < 0.05).

Standard error is presented within parentheses.

Despite the high TS and the summer emergence of new tiller generations of DG in the first summer under understory conditions, a severe decrease in TPD occurred during the second summer (Table 3). In tree

rows oriented N-S and full sunlight conditions, the specie exhibited significantly lower TPD compared to the first year.

Table 3

Adjusted means and standard errors for tiller population density (tiller m^{-2}) of *Dactylis glomerata* in both years of study (2019–2020 and 2020–2021) in two different conditions: Full sunlight and with tree (*E. Grandis x tereticornis*) rows in North-South (N-S) orientation.

Year	Tiller population density (tillers m^{-2}) for <i>Dactylis glomerata</i>				
	Dec	Jan	Feb	Mar	Apr
<i>Full sunlight</i>					
2019–2020	3017 (309) ^{1 A}	2317 (309) ^{AB}	2600 (309) ^{AB}	2333 (309) ^{AB}	1750 (309) ^{ABCD}
2020–2021	1850 (309) ^{ABCD}	1083 (309) ^{BCDE}	1050 (309) ^{BCDE}	500 (309) ^{CDE}	450 (309) ^{DE}
<i>N-S</i>					
2019–2020	1933 (255) ^{AB}	1689 (255) ^{ABCD}	2622 (255) ^A	2944 (255) ^A	2633 (255) ^A
2020–2021	1889 (255) ^{ABCD}	1078 (255) ^{BCDE}	556 (255) ^{CDE}	233 (255) ^E	133 (255) ^E

¹The means sharing the same letters do not show significant differences for the triple interaction Year x Condition x Month by Tukey test ($P < 0.05$).

Standard error is presented within parentheses.

In all species and treatments, tillers formed in December represented more than 40% of those recorded in April, except in HL (Fig. 4). However, the dynamics of survival showed differences. While DG showed a remarkable emergence of new tillers even during the hottest period of the year, combined with a low mortality in all generations, FA showed a low contribution of new tillers in understory conditions during summer, and none of them survived. For HL, the complete mortality of all tillers in the understory condition determined that the specie would not persist into the second year (Fig. 4).

During the second summer of the study, a reduced tiller density was observed at the beginning of the summer period for DG in two treatments (full sunlight and tree rows-oriented N-S; Fig. 5). Subsequently, there was a high mortality in the first tiller generation and a limited emergence of new tiller generations, particularly in the N-S tree rows orientation. These factors explain the low TPD observed at the end of the summer (Table 3).

Appearance and mortality rates

The appearance and mortality rates of tillers presented different dynamics based on the species and treatments in the first year of study (Fig. 6). HL experienced the highest mortality rate in the experiment, in February and March under the influence of trees (Fig. 6). FA showed a high mortality rate under the trees, especially in the E-W row orientation in March and April. Mortality rate for DG under full sunlight was higher than under the trees throughout the period, the most significant differences occurring in February (Fig. 6).

DG showed the highest appearance rate among species. The higher value was detected during the month of February under the trees in the N-S row orientation, but it decreased towards the end of the period, while the values under full sunlight remained stable throughout the experiment. In the E-W row orientation the appearance of tillers was always inferior to the other treatments (Fig. 6).

Relationships between microclimatic conditions and tiller dynamics

Principal component analysis of the first year of study indicate that the influence of microclimatic conditions on tiller dynamics varies according each studied specie. In the case of HL, TS and TPD exhibited stronger associations with soil moisture, while for FA, these parameters showed higher correlation with PAR. Conversely, TDR demonstrated a robust and negative relationship with soil moisture (for HL) and with PAR (for FA). In contrast, DG demonstrated relatively modest responses to the prevailing microclimatic conditions, predominantly displaying a negative association of TPD and TS with both PAR and soil moisture.

Discussion

The hypothesis that the microclimatic conditions prevalent in SPS may reduce tiller mortality, and potentially stimulate tiller appearance in grasses better adapted to the shade, during the typical summer of subtropical climates was accepted. DG emerged as the specie with the best understory persistence in the first summer, attributable to the highest degree of tiller survival and appearance in relation to its own full sunlight performance and to the other species, especially when tree rows were N-S oriented. HL displayed high sensitivity to summer conditions, irrespective of the light conditions, experiencing a complete tiller mortality under tree influence but not in full sunlight. FA has better tiller survival and final tiller density in full sunlight, yet SPS increased tiller death, compromising its overall persistence. Despite the greater TPD for DG after the first summer, the challenging environmental conditions in the subsequent year compromised tiller survival, especially in the silvopastoral understory.

The study's findings align with the understanding that the spatial arrangement of trees in SPS plays a crucial role in influencing microclimate, which, in turn, affects the performance of understory forage species. Despite a substantial 20-meter distance between tree rows, the microclimatic conditions observed in the silvopastoral understory in both years of study can be considered highly challenging for the development of the forage species. Considering that the arboreal component consisted of Eucalyptus trees (perennial leaves) with approximately 30 m of height in this study, high PAR reductions

were observed in both understory conditions. The PAR transmissivity reached only 20% during the winter in E-W oriented tree rows compared to full sunlight treatment (100% of transmissivity). Additionally, soil moisture was consistently lower in understory conditions for most of the year, despite the difference was reduced during the absence of rain. N-S tree row orientation maintained the lowest level of water content in the soil throughout all the experiment, but all conditions presented a severe reduction in soil moisture during summer in both years. Soil temperature consistently remained higher in the full sunlight treatment throughout the experiment, with similar values observed between SPS conditions. These results align with the data obtained in the study by Karki and Goodman (2015) for mature trees, where the soil temperature was higher in full sunlight treatment during all the experiment, and soil moisture under the SPS was lower than in open pasture. In contrast to young trees, mature trees exhibit greater need for water and more extensive root systems to absorb the water available in the soil, generating higher water demand in SPS compared to open pasture (Karki and Goodman, 2015).

Irrespective of their genotypic characteristics for tolerating shade conditions, the three grass species studied in the understory showed lower TPD at the end of spring compared to those in full sunlight. It is well established that in shaded sites, where photosynthates are limited, tillering is diminished due to the preferential allocation of current photosynthate to existing tillers rather than to the development of new tiller buds (Belesky et al. 2006). In the present study, however, the factor determining greater summer persistence in the understory was not solely the quantity of tiller present at the end of spring, but rather the reduced senescence and the emergence of new tiller generations during the summer. Under tree canopies, DG exhibited the highest TS and maintained a consistently low TDR throughout the observation period in relation to the other treatments and to the other species. Notably, DG presented the highest tiller appearance in February, which was slightly superior in the N-S compared to E-W tree row orientation. As a consequence, DG ended the summer period with a greater tiller density than it had at the beginning of this season in the first year of study.

On the other hand, when exposed to full sunlight conditions, FA displayed the highest TPD both at the beginning and at the end of the summer period. Previous research have shown that the persistence of FA during summer in subtropical climates is primarily determined by the number of tillers present at the end of spring (Jáuregui et al. 2017). In this context, Duchini et al. (2018) demonstrated that for species with functional types classified as conservative growth strategy, such as FA, persistence is primarily determined by the survival of the existing tillers. However, for those classified as moderately exploitative, like DG, persistence is also influenced by the continuous appearance of new tillers. In the current study, the higher tiller density in FA at the end of spring, rather than the appearance of new tillers in the summer, played a pivotal role in contributing to its increased persistence under full sunlight. This finding aligns with previous research conducted in analogous climatic conditions (Jáuregui et al., 2017).

Despite exhibiting higher TS in full sunlight, FA showed lower TS and reduced tiller appearance during summer when cultivated under tree canopies, when compared to DG. As a shade-tolerant specie, DG exhibits the adaptive capacity to modulate its photosynthetic processes, ensuring optimal functionality under low-light conditions (Mercier et al. 2020). This inherent trait underscores the species' proficiency

for sustained survival over an extended period within the canopy's shaded environment. Alternatively, grasses with lower shade adaptation (classified as shade avoiders) exhibit increased partitioning preferentially to shoot at the expense of root biomass when exposed to low light environments (Solofondranohatra et al. 2021). In naturally dense communities, shoot elongation induced by low red/far-red light may confer a fitness advantage, enabling shade-avoiding grasses to adjust their growth patterns, optimize their exposure to sunlight, and rapidly dominate gaps in the canopy (Pierik et al., 2013). However, as elongation is frequently attained at the cost of leaf, tillers and root growth, shade avoidance may result in a reduction in the productivity in understored conditions. Indeed, the reduced root biomass could lead to a diminished capacity for these species to persist in situations where the soils exhibit low water availability, high temperatures, and low air humidity - conditions typically prevalent during the summer in the context of this study.

In the case of HL, a biennial specie, high tiller mortality rate was observed in all situations. In understory conditions, all tillers present at the end of spring died during the summer. Despite some studies suggesting its suitability for low-light environments (Olmos et al., 2011), this specie, with low tolerance for dry conditions, encountered challenges in thriving under severe soil water restriction during the summer in the tree understory. Nevertheless, a few tillers were able to survive only in the full sunlight (exhibiting higher soil moisture levels), potentially contributing to the species' persistence in the following year.

The investigation into the development of forage species under varying light conditions is a common focus in various studies, as light is widely acknowledged as the most limiting factor in silvopastoral systems. However, our study clearly demonstrates that the association between different microclimatic variables synchronously influences the response of crops in forest understories. Water is a primary factor for forage development, and this variable significantly decreased in understory conditions in both study years. From December to January in the N-S treatment, soil volumetric water content remained below 5% in the second year and averaged 15% in the first year. In this context of water restriction, Volaire and Lelièvre (2001) showed that DG exhibits a better ability for water uptake at very low soil water potentials and higher dehydration control than FA. In our conditions, DG showed a higher number of tillers at the end of the first summer compared to FA and HL in understory conditions, but in the second year we did not observe a similar performance. Probably, the association between shade tolerance and drought survival strategies allowed DG to persist better in the first year but was not sufficient for the following year.

Conclusions

The results showed that DG presented lower relative tiller mortality rate; and a greater tiller survival and appearance under conditions of decreased radiation and soil moisture generated by the trees. The effect was accentuated in N-S tree row orientation, due to the greater appearance of tillers during the month of February. For FA, the reduced amount of light accelerated the process of tiller mortality, while for HL, the dry summer conditions caused a greater mortality, a process that was increased by the presence of the

trees. Due to its resilience to adverse climatic conditions, DG appears as a promising specie for SPS in subtropical climates in terms of summer persistence.

Declarations

Author Contribution

JKF conceived and designed the research. CH, JKF, PB, CVG, JGT performed the field evaluations. CH, JKF, CVG, JGT wrote the main manuscript text. CH and PGB performed the statistical analysis. All authors reviewed the manuscript.

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Figures

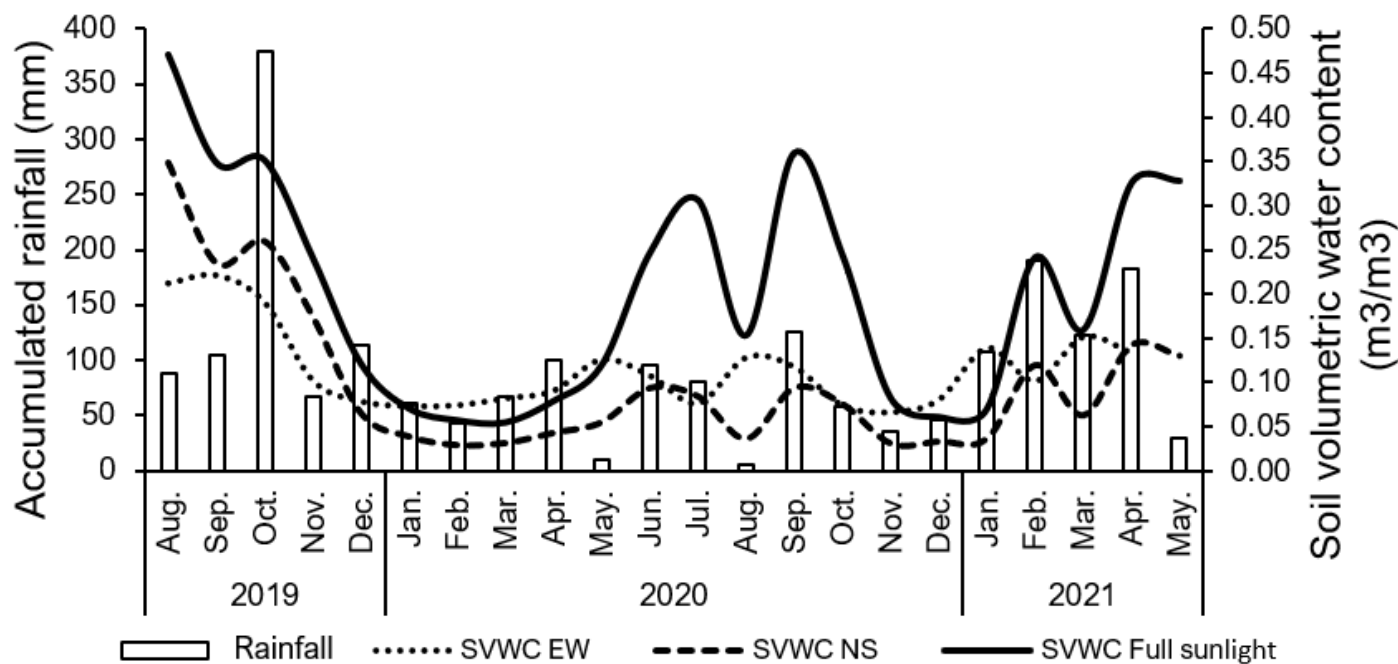


Figure 1

Accumulated rainfall (mm) in the experimental unit (white bars) under full sunlight condition and soil volumetric water content (SVWC, m³/m³) for full sunlight (continuous line), silvopastoral systems in East-West (dotted line) and North-South tree orientation (dashed line).

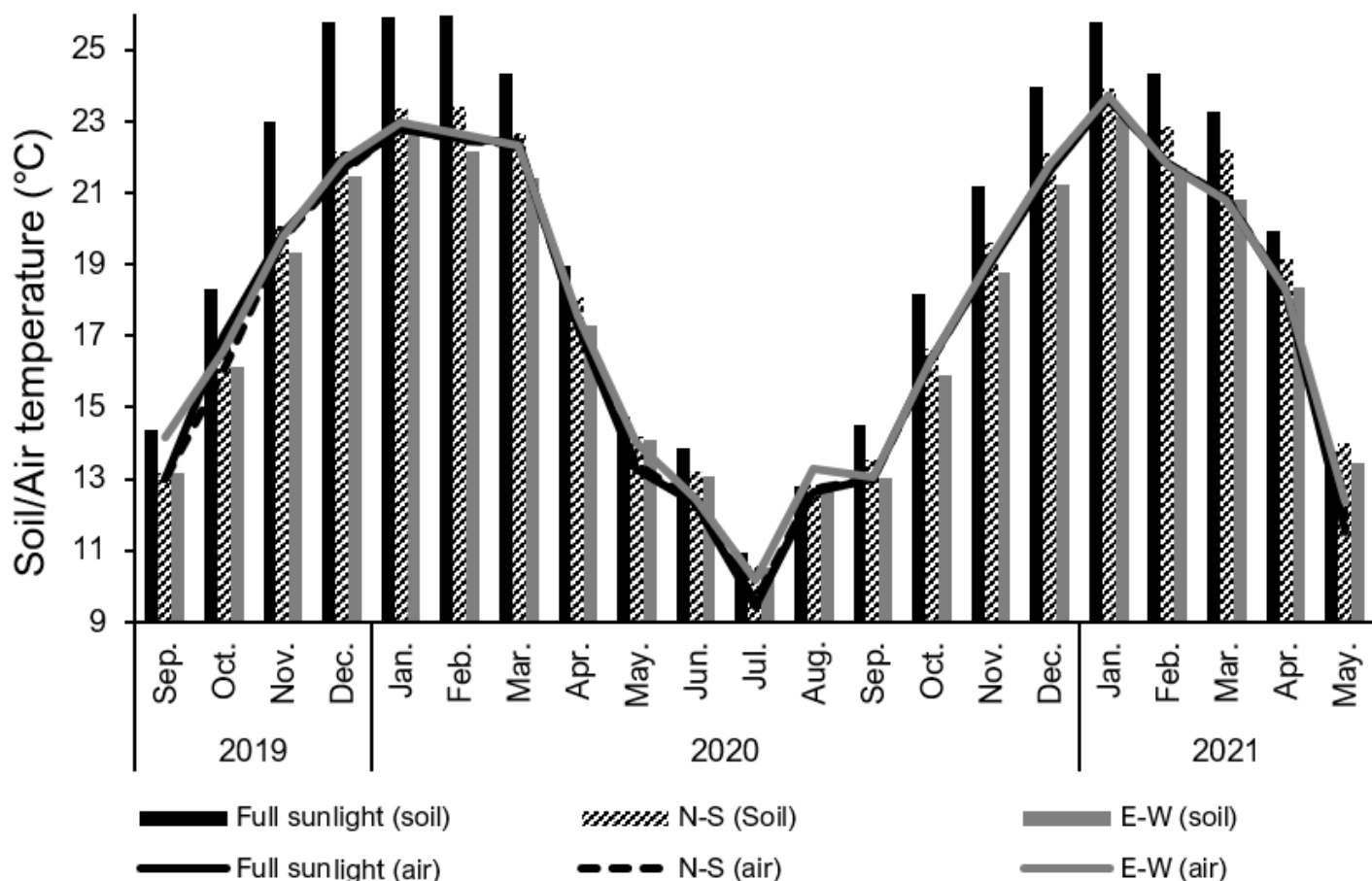


Figure 2

Monthly Average of Soil and Air temperature (°C) during the experimental period for the full sunlight (solid black bars and continuous line) and silvopastoral systems in East-West (solid gray bars and continuous gray line) and North-South tree orientation (dashed black bars and dashed black line).

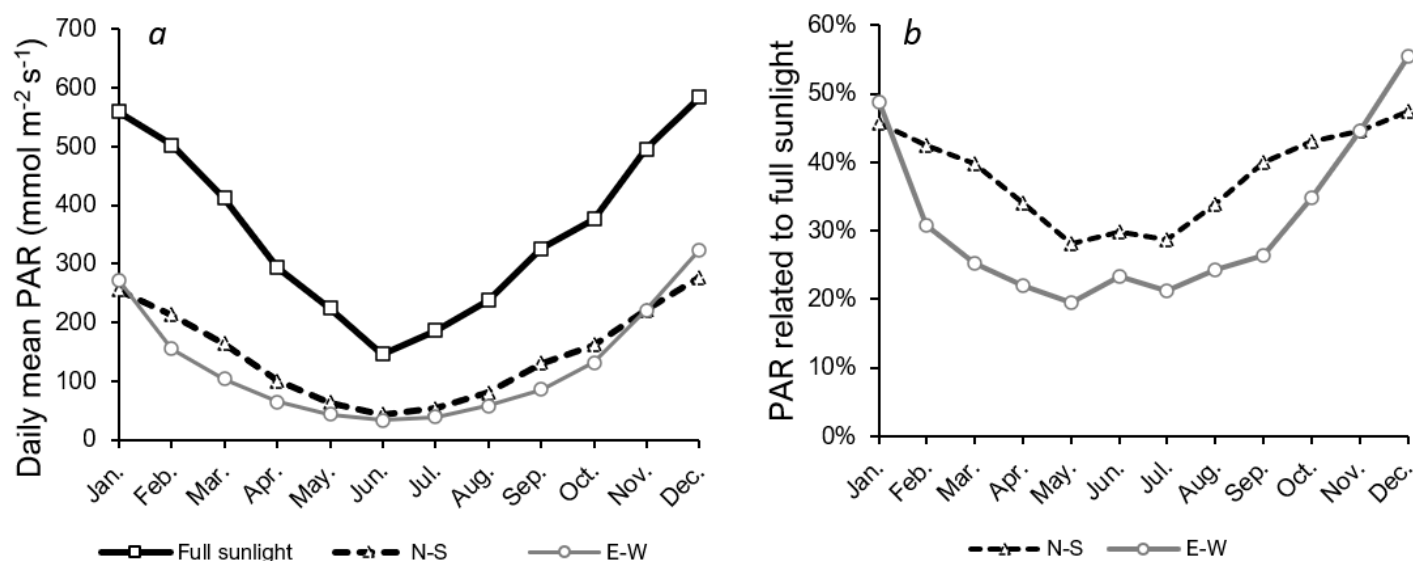


Figure 3

Monthly mean values of Photosynthetically Active Radiation (PAR) ($\text{mmol}/\text{m}^2/\text{s}$) (Fig. 3a) and relative values of PAR in silvopastoral systems with tree rows, oriented North-South and East-West in comparison to full sunlight (%) (Fig. 3b). Monthly PAR values represent an average for the years 2019, 2020, and 2021.

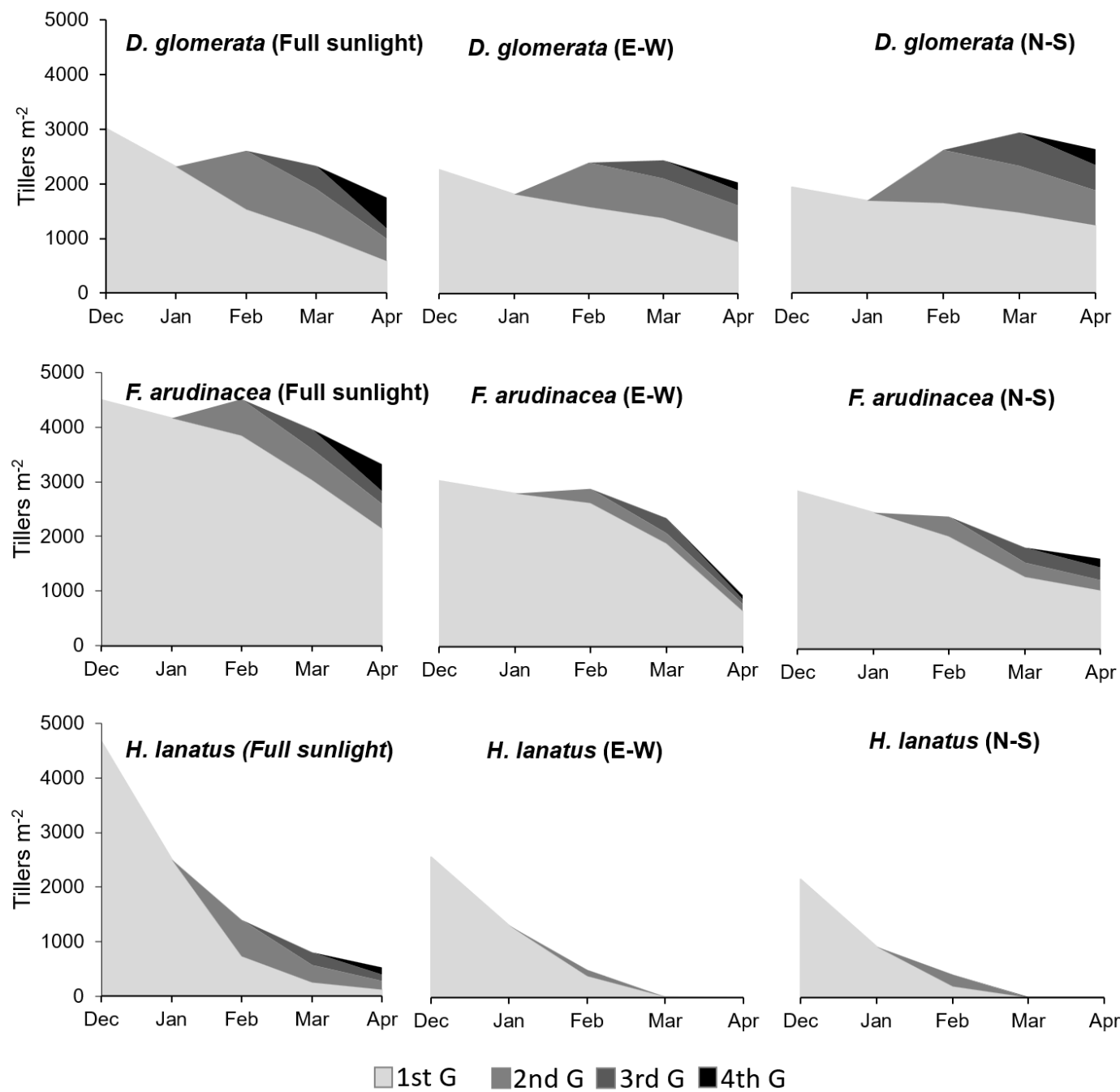


Figure 4

Tiller population density (TPD) of first, second, third and fourth generation (greyscale from lighter to darker; respectively) of *Dactylis glomerata*, *Festuca arundinacea* and *Holcus lanatus* from January to

April of the first year of the experiment in three different treatments: without trees (Full Sunlight) and with tree (*E. Grandis x tereticornis*) rows in two different orientations (North-South (NS) and East-West (EW)).

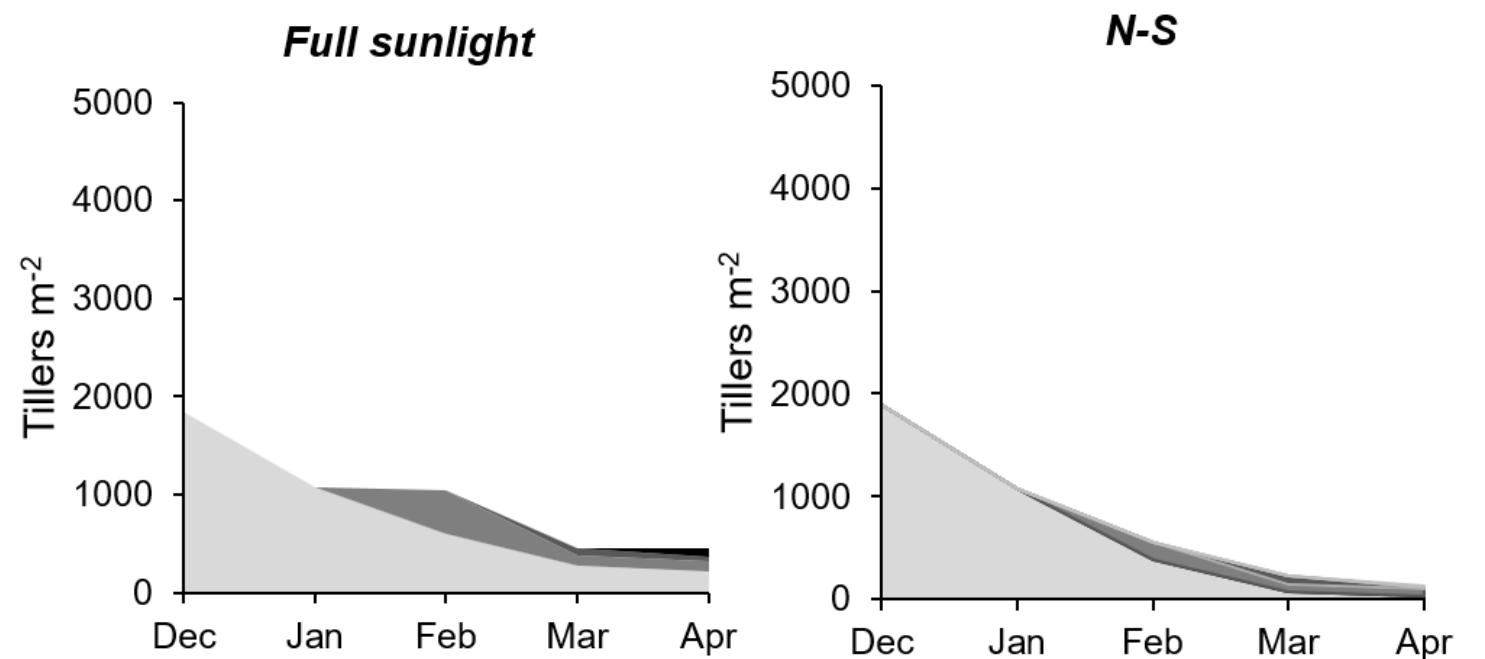


Figure 5

Tiller population density (TPD) of first, second, third and fourth generation (greyscale from lighter to darker respectively) of *Dactylis glomerata* from January to April of the second year of experiment in two different treatments: full sunlight and with tree (*E. Grandis x tereticornis*) rows in North-South (N-S) orientation.

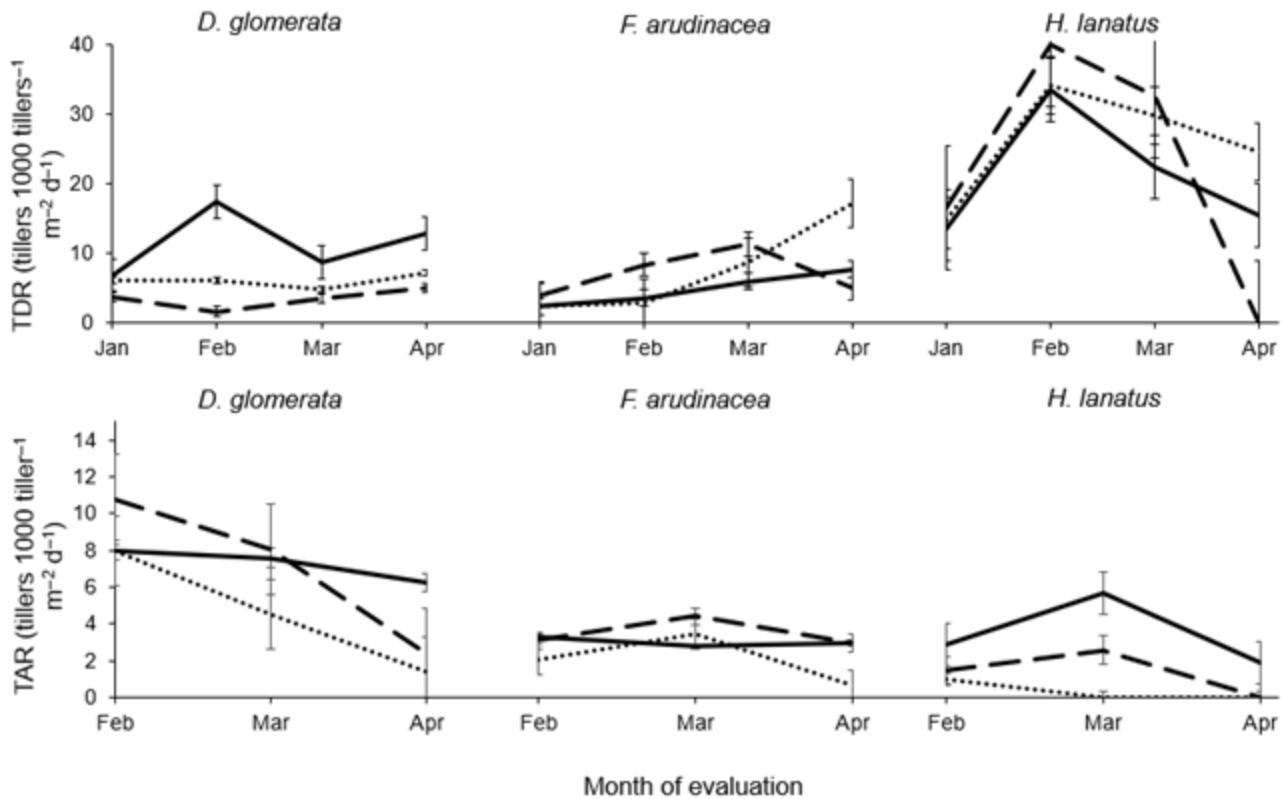


Figure 6

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

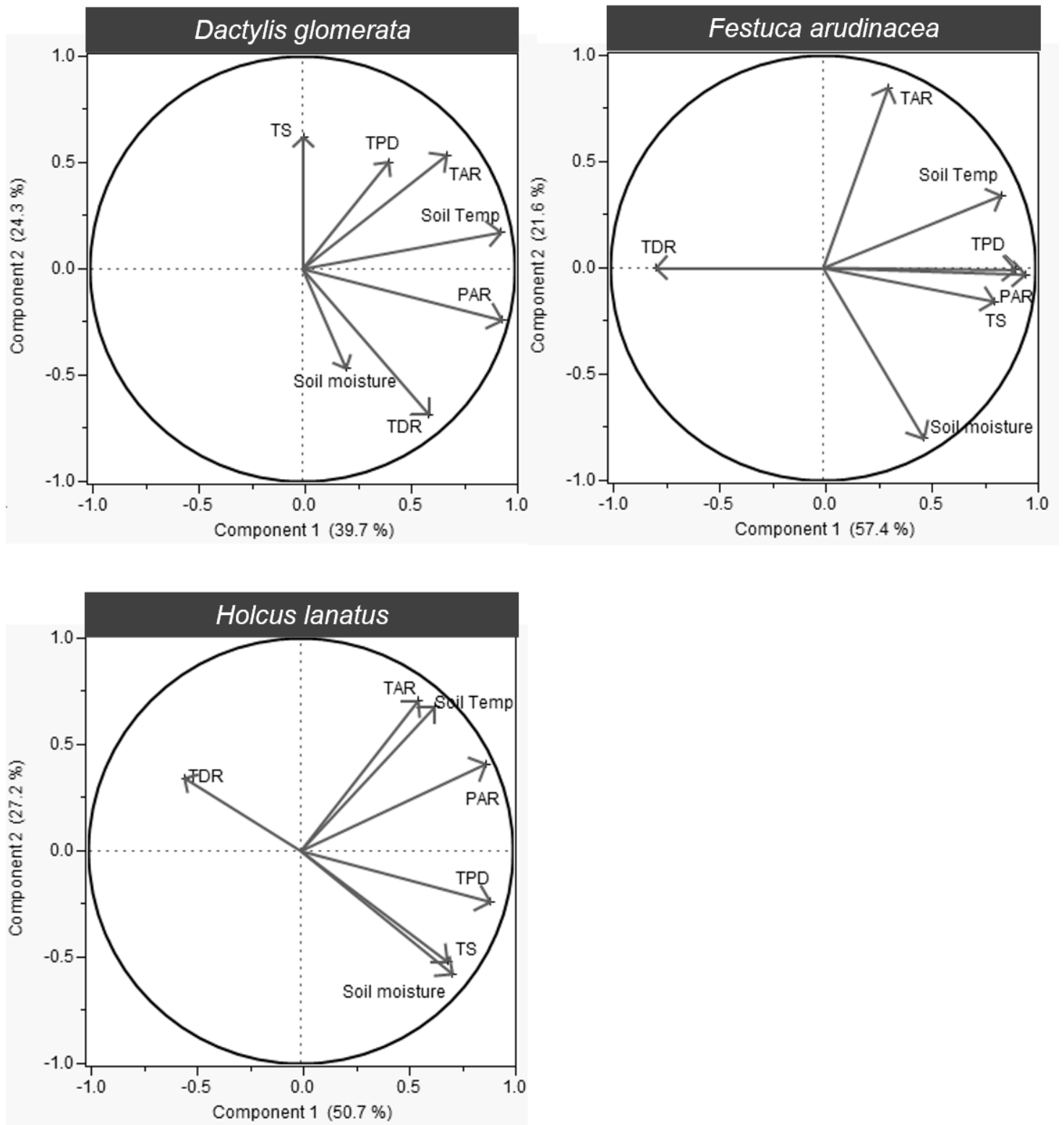


Figure 7

Principal component analysis for *Dactylis glomerata*, *Festuca arundinacea* and *Holcus lanatus* that characterizes the relationships exhibited by the variables tiller population density (TPD), tiller survival (TS), tiller death rate (TDR) and tiller appearance rate (TAR) with the environmental parameters photosynthetic active radiation (PAR), mean soil temperature (Soil Temp), and Soil moisture. The

proportion of the total variance (%) explained by each principal component is presented between brackets.