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Evaluation of photosynthetic variables of *Brachiaria brizantha* under eucalyptus canopies in a livestock-forestry integration system

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Abstract. Livestock sector generates significant environmental impacts despite its global economic importance. The current challenge is to find sustainable ways of boosting this sector, while mitigating the negative impacts of this activity. In Brazil, degraded pastures are common because of inadequate management, damaging the soil. Integrated livestock-forestry (ILF) systems combine forest species and pastures in the area, incorporating elements of sustainability. In this system, the tree species is related to the productivity of the pasture, as the shade generated by the canopy creates different light conditions, influencing the photosynthetic activity of the forage. The aim of this study was to evaluate the influence of the luminosity of the understory of an ILF system on the photosynthetic activity of the forage species *Brachiaria brizantha* in the Atlantic Forest region of Brazil. Transient chlorophyll a fluorescence and chlorophyll concentration were analysed in forage plants grown in consortium with *Eucalyptus urograndis* (Clone 1407), with spacing of 4m between trees and 24m between tree rows. Two treatments were established based on light conditions: the control treatment, corresponding to the condition with the highest light corresponded to the pasture row, and the treatment with the lowest light, corresponding to the area under the canopy. The results show that the low light supplied to the forage plant, during the experiment period, under eucalyptus canopies, promotes changes in the intensity of chlorophyll a fluorescence and chlorophyll concentration, indicating low efficiency of the electron transport chain and changes in leaf nitrogen content, due to a possible stress situation.

Key words: cattle, forage, light stress, photosynthesis, silvopastoral system.

INTRODUCTION

Livestock activity represents one of the oldest practices in the Brazilian economic scenario. This activity plays a crucial role in the global economy and is a catalyst for economic development, as it contributes significantly to the Gross Domestic Product (GDP) of different nations (Yitbarek, 2019). According to the US Department of Agriculture, global production in 2024 is expected to be equivalent to approximately 59 million tons of beef, with Brazilian production increasing by 3% to a record 10.8 million tons (USDA, 2023).

Despite being essential to the country's economic sector, it is inextricably linked to environmental impacts. A distinctive feature of livestock in Brazil is grazing, which is the most practical and economical way of feeding cattle (Carvalho et al., 2017). Due to this practice, Brazil has extensive grazing areas, equivalent to approximately 159 million hectares, and it is estimated that about 50% of these areas have some degree of degradation (IBGE, 2017).

Rangeland degradation results from the interaction of several factors, such as inadequate management, leading to soil erosion and the leaching and volatilization of nutrients without replacement (Peron & Evangelista, 2004). The search for sustainable approaches that mitigate the negative impacts associated with livestock farming, such as conservation practices, is therefore of great importance.

Conservation practices consist of all the techniques used to increase resilience or reduce the forces of the erosion process, with the aim of preserving and conserving natural resources (Bertoni & Lombardi Neto, 2017). These practices include the integrated livestock-forestry system (ILF), in which trees can be used simultaneously with the establishment of pastures, creating a microclimate that increases thermal comfort for the animals (Gil et al., 2015; Pezzopane et al., 2019).

The microclimate created by the introduction of the tree component into the system, affects not only the health of the animals, but also on the health of the pasture, since the shading created by the tree canopy affects the brightness available to the species growing under the canopy, influencing the morphophysiological aspects of the pastures (Paciullo et al., 2008). In tropical climates, a transmission of 65% of the photosynthetically active radiation, used for the process of photosynthesis can be considered as a threshold to produce tropical forage in integrated systems with trees (Paciullo et al., 2011). Silvopastoral systems with smaller spacings and higher tree densities have lower rates of photosynthetically active radiation on the leaf surface and leaf photosynthesis (Santos Neto et al., 2023).

The presence of chlorophyll in leaf cells is fundamental for the efficient realisation of photosynthesis and chlorophyll loss is associated with environmental stresses (Zhao et al., 2016). The Soil Plant Analysis Development (SPAD) chlorophyll meter provides a rapid and non-destructive approach that enables users to measure relative chlorophyll concentration content in the field of leaves, this is especially important in determining nitrogen status (Yuan et al., 2016). The content of chlorophyll in the leaves is highly correlated with crude protein, the most studied characteristic in terms of the chemical composition and nutritional value of the pasture (Liu et al., 2017).

Brachiaria species are the most important forage plants on the pastures of Brazil. They have a high nutritional value and can meet the feed requirements of cattle raised in the tropics, as they are very drought-resistant, easy to propagate and adapted to the tropical climate of Brazil (Kono et al., 2022).

Brachiaria brizantha, also known as *Urochloa brizantha,* is a tropical C_4 grass classified as medium shade tolerant and known for its rapid growth potential. It plays an important role as a pasture worldwide, occupying large areas in tropical and subtropical regions, especially on soils with limited fertility (Martins et al., 2014). Although *B. brizantha* can adapt its photosynthetic behaviour in response to shade by lowering its light compensation points, its photosynthetic capacity can be reduced under permanent shade (Dias-Filho, 2002).

In this context, the aim of this study is to evaluate the effects of brightness in the understory of an ILF system based on the photosynthetic activity of the species *Brachiaria brizantha* in an Atlantic Forest. This analysis will help to understand of the potential benefits of this sustainable approach that integrates environmental, economic, and social aspects in the management of Brazilian livestock production.

MATERIALS AND METHODS

The experiment was conducted at the Santa Mônica Experimental Field (CESM), which belongs to the Brazilian Dairy Cattle Research Corporation, located in the municipality of Valença in the state of Rio de Janeiro. The data was collected in May 2023 at geographical coordinates 22°21'31"S and 43°41'42"W. The climate in the region was classified as Cwa according to the Köppen classification and is characterized by a dry winter and a rainy summer. The experimental area comprised 1 ha of a 4-year-old livestock-forest system in hilly topography, with gradients of up to 20% and an average altitude of 356 m.

The soil of the areas was classified as Red Yellow Argisol of clayey texture (Santos et al., 2018). Average soil chemical properties in the area were: pH (water), 5.8; organic matter (OM), 2.91 dag kg^{-1}; P(Mehlich 1), 7.05 mg dm^{-3}; K(Mehlich 1), 123 mg dm^{-3}; Ca, 2.0 cmolc dm^{-1}; Mg, 1.72 cmolc dm^{-3}; Al, 0.17 cmolc dm^{-3}; H + Al, 3.80 cmolc dm^{-3}.

The climatological data were collected by the National Meteorological Institute (INMET) for the Valença-RJ weather station. The data obtained were then processed using EXCEL software and the average values for temperature (°C), relative humidity (%), precipitation (mm) and radiation (KJ m^{-2}) were analysed (Fig. 1).

The analyses were carried out in an integrated livestock-forest system area (ILF), with *Brachiaria brizantha* in combination with a 4-year-old clone of the hybrid *Eucalyptus urophylla* x *E. grandis* (Clone 1407). The trees were planted in contour lines and arranged in single rows 25 meters apart and 4 meters between trees, totalling 100 trees per hectare. The average height of the trees during the study was 20 meters, and the area occupied by the forest component was 2.85 m^2 ha^{-1}.

The measurements were taken in the morning because, according to Santos Neto et al. (2023), the photosynthetically active radiation on the leaf surface has a significant difference in the silvopastoral system according to the time of day, where the highest rates are observed between 9 am and 12 pm.

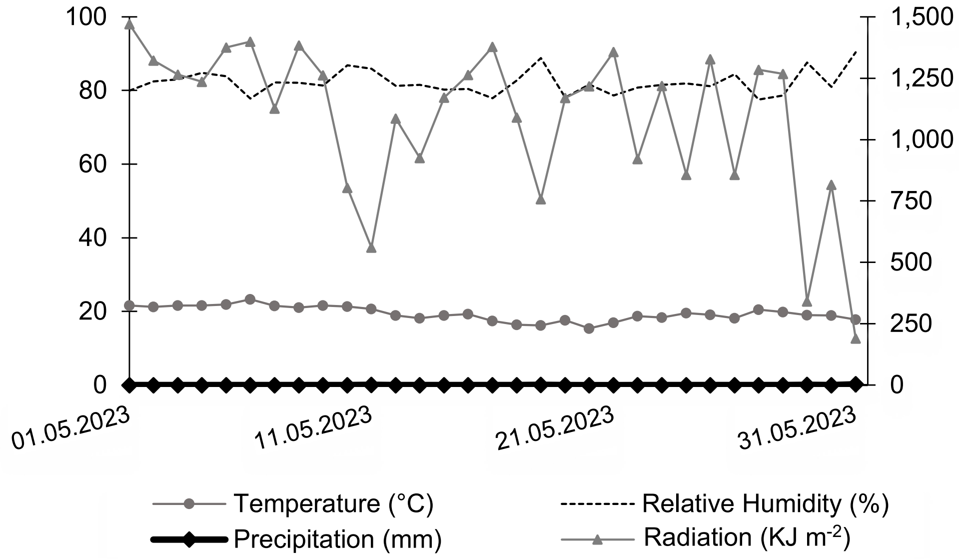

Figure 1. Climatological data provided by the Valença-RJ weather station during measurements in May, collected by the National Meteorological Institute (INMET).

The treatments were conducted under to two lighting conditions. The control treatment (T1) corresponded to the highest light intensity corresponded to the pasture rows. In contrast, the treatment in the tree row (T2) corresponded to the lowest light incidence, observed in the region under the canopy of the eucalyptus trees. The five points where the analyses were carried out were chosen at random, in such a way that for T1 it was the areas equidistant between the tree rows, and for T2 it was the areas equidistant between the trees (Fig. 2).

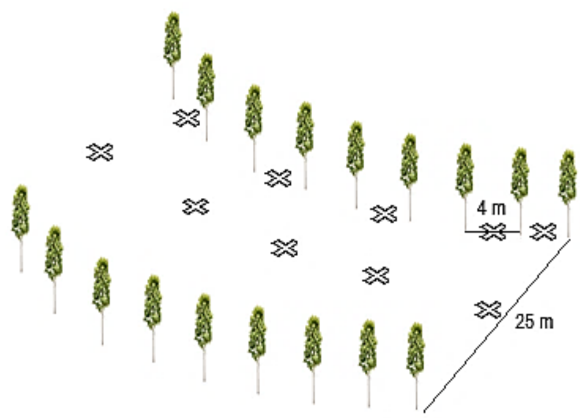

Figure 2. Delineation of the data collection points in relation to the eucalyptus (*Eucalyptus urophylla* x *E. grandis)* trees in the experimental area of the integrated livestock-forestry (ILF) system in Valença-RJ.

To analyse transient fluorescence of chlorophyll *a*, 3 *B. brizantha* leaves were randomly selected from each point (totalling 15 repetitions for each treatment). The selected leaves were previously adapted to the absence of light for 30 minutes using a closed clip.A Handy PEA portable fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK) was used for the measurements and the parameters were calculated according to the methodology proposed by Strasser & Strasser (1995) and Tsimilli-Michael & Strasser (2008). In addition, the data of the obtained parameters were subjected to analysis of variance (ANAVA) with Tukey at 5%, which was performed using the SISVAR® software.

At the same time, the relative chlorophyll content was measured using a Soil Plant Analysis Development (SPAD) chlorophyll meter model Minolta SPAD-502, avoiding the central vein. The SPAD index was measured using the average of three readings on the most expanded leaves of *B. brizantha* chosen at random for each point (5 repetitions for each treatment). As with the chlorophyll a fluorescence parameters, the data obtained from the SPAD analysis were subjected to statistical analysis using Tuckey at 5%.

RESULTS AND DISCUSSION

Different light intensities usually trigger morphological and physiological changes in plants, whereby the degree of adaptation is determined by the characteristics of the respective species in interaction with its environment (Scalon et al., 2003; Kleinwächter & Selmar, 2015). In recent years, photoinhibition has been studied using various approaches, with chlorophyll fluorescence being the most used approach to better understand the photosynthetic process in plants (Murchie & Lawson, 2013).

The data obtained by analysing the transient chlorophyll *a* fluorescence indicate that the reduction in light intensity of the understory caused by the shading induced by the eucalyptus trees caused significant changes in the electron transport chain of the *B. brizantha* forage, as shown in Fig. 3, for the forage grown in the tree rows.

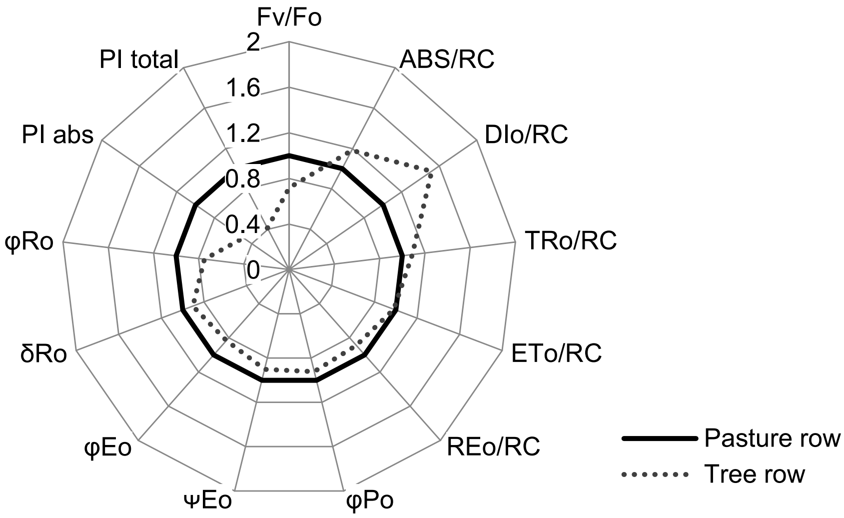

Figure 3. Transient chlorophyll *a* fluorescence parameters of brachiaria (*B. brizantha*) when cultivated in the pasture row and in the tree row in an integrated livestock-forestry (ILF) system in the Atlantic Forest region of Valença-RJ.

The ratio between variable fluorescence and minimum fluorescence (Fv/Fo), which corresponds to the effective quantum yield of photochemical energy conversion, showed a lower rate in the treatment corresponding to the growth line compared to the normalized one, indicating a decreasing in the maximum efficiency of the photochemical process and a lower activity of photosystem II (PSII).

As for yields and flux ratios, the maximum primary photochemical quantum yield (φPo) was lower than the normalized level, as was the probability of a trapped exciton moving an electron in the electron transport chain after Quinone A (ΨEo) and the quantum yield of electron transport from Quinone A (Q_A) to the electron acceptor intersystem (φEo), indicating a failure in PSII.

The quantum yield of reduction of final photosystem I (PSI) electron acceptors per absorbed photon (φRo) and the efficiency with which an electron can move from the reduced intersystem electron acceptors to the final photosystem I (δRo) electron acceptors showed lower values than the control, demonstrating the power efficiency of electron transport between the two photosystems and, consequently lower success in the formation of NADPH, which impairs carbon fixation.

Regarding the specific flow parameters or activities expressed per reaction centre, the energy transport starts with the absorption (ABS) of light by the antenna pigments of PSII and ends with the reduction of the final electron acceptors on the electron acceptor side of PSI (RE) (Yusuf et al., 2010). The absorbance flux per reaction centre (ABS/RC) in the T2 treatment was statistically different from the control with an increase of 15% (Table 1). Similarly, the energy dissipation per reaction centre (DIo/RC) which corresponds to the total excitation energy not absorbed by the reaction centre, was also 34% higher than in the control treatment.

Table 1. Chlorophyll *a* fluorescence parameters analysed in brachiaria (*B. brizantha*) when cultivated in the pasture row and in the tree row in an integrated livestock-forestry (ILF) system in the Atlantic Forest region of Valença-RJ

Treatment	ABS/RC	DI_0/RC	TR_0/RC	ET_0/RC	RE_0/RC	PIabs	PItotal
T1	2.749 b	0.593 b	2.157 b	1.163 a	0.506 a	1.550 a	1.192 a
T2	3.249 a	0.900 a	2.349 a	1.141 a	0.449 b	0.758 b	0.491 b

Equal letters in the same column do not differ statistically by Tukey's test at 5%. Values represent the average of $n = 5$.

Environmental fluctuations lead to changes in the biochemical, morphological, and physiological characteristics of the plant, corresponding to its phenotypic plasticity, as a form of adaptation (Schlichting, 1986). The higher ABS/RC index of T2 could be related to a metabolic adaptation that causes an increase in the leaf area of the plant to intercept more light radiation to compensate for a stressful situation with less light than the species needs, a phenomenon known as hormesis (Vargas-Hernandez et al., 2017). Although T2 showed greater absorption, it also showed greater dissipation, not conserving the energy obtained and affecting the reduction of PSI. The flow of energy absorbed by the reaction centre (TRo/RC) was also 8% higher when compared to the control.

The flow of electron transport beyond Q_A per active reaction centre, represented by (ETo/RC) did not differ from the normalized value, while the specific flow of electrons capable of reducing the final electron acceptors in the electron acceptor portion of PSI per active reaction centre (REo/RC) remained 11% lower than the normalized value impaired NADPH production.

In terms of performance indices, the total performance index (PIabs) associated with conservation of energy from the photons absorbed by the PSII to the reduction of the electron acceptors between the systems, T2 was 51% lower than normalization, confirming the low efficiency of PSII. Similarly, the performance index up to the last electron acceptors of PSI (PItotal) was also 59% lower than in the pasture row treatment, indicating a complication in electron transport in the intersystem and showing that this species has little light available, which affects its growth and proper development.

According to the SPAD index measurements (Fig. 4), treatment T1 had a 23% higher chlorophyll content than treatment T2. The SPAD index helps to evaluate the nutritional status of the plants, as there is a positive correlation between the SPAD value and the nitrogen concentration in the leaves of the grasses (Zotarelli et al., 2003). As shown by Costa et al. (2009), an increase in nitrogen concentration leads to a linear increase in chlorophyll production (SPAD index) in *Brachiaria brizantha* cultivars.

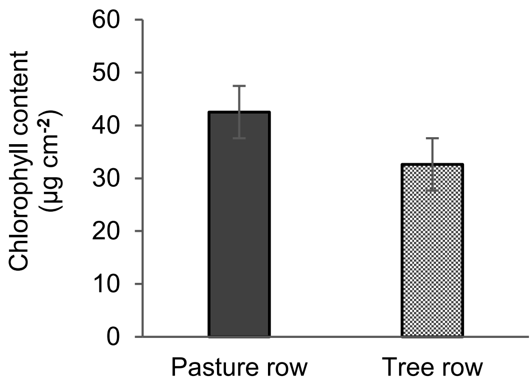

Figure 4. Chlorophyll concentration of brachiaria (*B. brizantha*) when grown in the pasture row and in the tree row in an integrated livestock-forestry (ILF) system in the Atlantic Forest region of Valença-RJ.

For the period analysed, which has low temperature indices, significant variations in radiation and no precipitation, stress is visible between the eucalyptus trees, where the canopies create a permanent shade for the forage plants (Fig. 5). This confirms corroborating the idea that the current spacing between the trees leads to intense shading, which significantly reduces the availability of direct sunlight in the understory and creates an unfavourable environment for the growth and development of the understory.

Figure 5. Abiotic stress generated by shading in brachiaria forage plants of brachiaria (*B. brizantha)* when cultivated when cultivated in the tree row in an integrated livestock-forestry (ILF) system in the Atlantic Forest region of Valença-RJ.

As also found by Santos et al. (2023), the ILF system significantly reduces photosynthetically active radiation (PAR) through tree density and consequently reduces forage production and dry matter yield, which is a disadvantage for a system whose main objective is livestock production. In a similar study carried out in a silvopastoral system composed of eucalyptus and *Brachiaria brizantha*, Santos et al. (2016) found that for every 1% reduction in photosynthetically active radiation there was a 1.35% decrease in forage dry mass.

CONCLUSIONS

The physiological response of *B. brizantha* to shading by eucalyptus trees grown in an ILF system in a Brazilian Atlantic Forest was found to be less efficient. The lighting conditions under the eucalyptus canopies promoted significant changes in the intensity of chlorophyll *a* fluorescence, with a low efficiency in the electron transport chain, indicating a possible stress situation, during the experimental period. A reduction in chlorophyll content was also observed, indicating direct effects on the photosynthetic potential of the forage plant.

These results indicate the crucial importance of light availability for photosynthetic performance and consequently for the growth and development of *B. brizantha*. The limitation of light due to the current spacing between eucalyptus trees used in cultivation may limit the biomass production of the forage and affect its quality and nutritional value as livestock feed.

Consequently, there is a clear need to manage the forest component by thinning to increase the light transmission to the understory. This intervention is essential to optimise the environmental conditions and promote the healthy growth of *B. brizantha*, thus ensuring the sustainability and productivity of the ILF system.

In addition, it is important to note that further studies are being carried out to analyse how the edaphic fauna and soil aggregation in this region interact with light conditions, affecting not only the photosynthetic performance of the vegetation, but also the overall quality of the ecosystem. Understanding these complex interactions is key to developing integrated management strategies that promote not only healthy forage growth, but also ecosystem health and resilience.

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