Differential responses of yield and shoot traits of five tropical grasses to N and distance to trees in silvopastoral systems

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Abstract

Light intensity and nitrogen (N) availability are important factors influencing the growth of C_4 forage species. Trade-offs may occur in the adaptive responses of species to shading and N inputs, and functional shoot traits can help to explain the consequences of these responses for species performance. Our objective was to gain understanding of the mechanisms between traits of five C_4 perennial grasses determining above-ground dry matter yield (DMY) when both resources, light and N, vary. Forage grasses were grown in six shading conditions (full sunlight vs. five positions between *Eucalyptus dunnii* rows) with two N levels (0 vs. 300 kg N $ha^{-1}year^{-1}$) and clipped when the canopy reached 95% light interception. Path analysis was used to explore the relationship between DMY, shading levels, N nutrition index and shoot traits. Dry matter yield increased between 126 to 569 g dry matter m^{-2} with N fertilization. Nitrogen nutrition index was the most important predictor for determining DMY followed by shading level. Increased shading reduced DMY by 9.5 g DM m^{-2} for each 1% of increase in shading. DMY was also modulated by shoot traits such as specific leaf area and leaf area index, but with different responses according to species, highlighting different strategies to cope with changes in light and N availability.

Introduction

Functional shoot traits can explain how different species respond to resource variation and the consequences for species performance (Schellberg and Pontes 2012). For instance, the relationship between functional traits and environmental factors led to identification of species strategies, of which a major one is related to resource-use, contrasting exploitative vs. conservative plant types (Wright et al. 2004). Three leaf traits, leaf N content (LNC), specific leaf area (SLA) and leaf dry matter content (LDMC), have stood out in the characterization of these two strategies (Garnier et al. 2004), as well as for understanding the role of species for ecosystem functioning and productivity (Pontes et al. 2007).

Studies have also found other important axes of variation in the relationship between functional traits and environmental factors, which is based on plant size, such as leaf area (LA) and plant height (Westoby 1998). The effect of resources such as N and light may be highly size-dependent because plants become increasingly inefficient as they grow. Self-shading and allocation of growth to structural components (Paciullo et al. 2016) reduce leaf production, which results in lower yield and nutritive value of grass species.

Light intensity and N availability seem to be the most important factors influencing growth of C_4 forage species, particularly in silvopastoral systems (Paciullo et al. 2016). Few studies have combined these two factors to explore the role of intraspecific trait variability in productivity, and their pathways on DMY remain unclear. Quantifying the link between the environment and plant functional traits is important to understanding the consequences of changes if resource availability, but such studies rarely extend into the cultivated C_4 species. Our objective was to understand the mechanisms relating shoot traits to above-ground DMY production when both light and N vary. We analyze the role of light restriction, according to the distance from trees, N availability and shoot traits on DMY of five C_4 grasses.

Methods

The experiment was located at the Rural Development Institute of Paraná – IAPAR-Emater, Ponta Grossa-PR (25°07’22’’ S, 50°03’01’’ W, 880 m a.s.l.), Brazil. The local climate is subtropical humid, with frequent occurrences of frost. Mean annual temperature was 19.3 ºC and annual precipitation was 1681 mm. The soil was Rhodic Hapludox (Oxisoil), with 531 g kg^{-1} of sand, 168 g kg^{-1} of silt, and 301 g kg^{-1} of clay in the open field, and 492 g kg^{-1} was sand, 162 g kg^{-1} was silt, and 346 g kg^{-1} was clay in the forested experimental area. For both areas, initial organic matter was 37 g kg^{-1} and average pH was 5.2. The study included five perennial grasses that are widely used for Brazilian livestock: *Axonopus catharinensis* (Ac), *Cynodon spp.* hybrid Tifton 85 (Cs), *Hemarthria altissima* cv. Flórida (Ha), *Megathyrsus maximus* cv. Aruana (Mm) and *Urochloa brizantha* cv. Marandu (Ub). In 2010, seeds (Mm, Ub) and seedlings (Ac, Cs, Ha) of these species were planted in pure stands in two side-by-side experiments, i.e., in the full sun (4.5 m² plots) and under *Eucalyptus dunnii* trees (100 m² plots). At the beginning of the growing period, liming, P_2O_5 and K_2O were applied according to soil analyses to ensure no limitation to plant growth. *Eucalyptus* trees were planted in 2007 (267 trees ha^{-1}) in a double-row arrangement with 3 m between trees within rows and 4 m between rows.

Data used in the current study represent well-established grass stands measured from October 2011 until May 2012. In addition to the species, the impact of two levels of N fertilizer (0 and 300 kg N $ha^{-1}year^{-1}$) and positions in relation to the trees were evaluated, with three replicates. Under trees, pastures were sampled at 2, 4, 10, 16 and 18 m (positions 1 until 5, respectively) from the trees. The full sun system was considered as the sixth position (P6).

The plots were mechanically cut when light interception (LI) of by the swards reached 95% on average. Canopy height (H) was determined at harvest, and 50% of this height was harvested. Dry matter yield above cutting height was determined by clipping herbage in 0.25 m^2 per plot and position. Annual DMY was calculated as the sum of mass from all cuts between 18 October 2011 and 28 May 2012. Shoot traits were assessed in 10 tillers collected at random in each plot and position, in January 2012, when LI reached 95%. Sheath length (SL) and number of leaves (NL) were measured first. Leaf length (LL), fresh mass and LA of the youngest fully expanded leaf lamina of each tiller were measured after tiller rehydration. Laminas were oven dried at 60 ºC for 48 h, weighed and LDMC and SLA were calculated. Tiller density (TD) was measured by counting the number of tillers in 0.0625 m^2. Leaf area index (LAI) was calculated as: LA × NL × TD. Samples of laminas and herbage harvested for DMY were analyzed via near-infrared reflectance spectroscopy for crude protein (CP) concentration. Leaf N content was calculated as CP/6.25 and LNC on fresh mass basis (LNCF) was calculated as LNC×LDMC. Nitrogen nutrition status was estimated using the NNI method (Lemaire and Gastal 1997).

DMY was analyzed with a mixed model including species, N fertilization, position, and their interactions as fixed effects. Block, whole plot and strips were included as a random effect. Model with DMY as a dependent variable was performed using the percentage of shading at each position, LAI, H, leaf weight ratio (LWR), LNCF, NNI and SLA as independent variable, as well as the factor position and interactions of these variables with species factors. The best model was defined using stepwise model selection with the "stepAIC" function of the "MASS" package (Venables and Ripley 2002) for R (R Core Team 2022). All statistical analyses were performed with R within the RStudio IDE.

Results and Discussion

Average PAR intensity in full sun between 06:00 and 18:00 was 403 W m^{-2} and shading levels under trees ranged from 21±2.1% in P3 to 50±4.0% in P1. The species × N levels × position interaction was significant ($P<0.05$) for DMY. All species showed an increase in DMY (Table 1) with N fertilization, except Ha at P2, P3 and P6. No-significant differences were observed on DMY between full sun and moderate shading (as in P3 position) for any species and N levels. This result agrees with Pang et al. (2017) which showed that for most forage species, yield can be maintained or improved under moderate shading compared to forages grown without trees when tree root competition is minimized.

Path analysis highlighted a positive and significant effect of N, mediated by NNI, and a negative effect of shading on DMY (Figure 1). LNC is integral to the proteins of the photosynthetic machinery, especially rubisco, which is responsible for the first step in CO_2 fixation (Wright et al. 2004). Light is an important environmental factor influencing plant growth, nutrient uptake, and yield, as well as the response of yield to N application (Paciullo et al. 2016). The fact that the final model included both the level of shading and position (Figure 1) indicate that other direct mechanisms related to position relative to the trees but unrelated to shading, were important in determining production. It is possible that tree-grass interactions in the soil, like competition for water or interference between roots were behind the effect of position beyond shading. Specific leaf area was the third most important variable explaining DMY (Figure 1). This is an important trait determining a plant's relative growth rate, because by increasing the area of a given unit of leaf biomass, the interception of light is increased, especially under low-light conditions (Poorter et al. 2009). The coordination between SLA and LNC makes it possible to optimize the amount of leaf organic nitrogen per unit of area and so the maximization of light-use efficiency (Paciullo et al. 2016). TD and SL seem to be the most important shoot traits, related to light capture, affecting LAI and H, respectively.

Table 1. Annual dry matter yield (g m^{-2}) for each species, nitrogen level (0 and 300 kg N $ha^{-1}year^{-1}$, N0 and N300, respectively) and position between tree rows and at full sun (P6). Positions 1 and 5 were situated closest to the tree's rows; P3, was a central position between trees rows; and P2 and P4, the intermediate positions.

	P1	P2	P3	P4	P5	P6
				N0		
Ac	302±151.1a	449±151.1a	660±151.1a	618±151.1a	358±151.1a	582±123.4a
Cs	318±123.4b	605±123.4ab	549±123.4ab	338±123.4b	253±123.4b	821±123.4a
Ha	500±123.4b	953±123.4b	1381±123.4a	690±123.4b	525±123.4b	962±123.4ab
Mm	320±151.1a	358±151.1a	565±151.1a	601±151.1a	425±151.1a	756±123.4a
Ub	444±123.4bc	530±123.4bc	672±123.4ab	440±123.4bc	298±123.4c	1043±123.4a
				N300		
Ac	533±151.1b	824±151.1ab	1052±151.1a	924±151.1ab	583±151.1b	1271±123.4a
Cs	736±123.4d	1044±123.4bc	1281±123.4ab	927±123.4bcd	641±123.4d	1667±123.4a
Ha	639±123.4b	925±123.4ab	1250±123.4a	1051±123.4a	991±144.9ab	911±123.4ab
Mm	573±123.4b	1001±123.4a	1193±123.4a	1167±123.4a	875±123.4b	1371±123.4a
Ub	1115±123.4ab	1207±123.4ab	1348±123.4a	1363±123.4a	923±144.9b	1119±123.4ab

Within rows, means followed by the same letter are not significantly different according to Tukey test ($P < 0.10$).

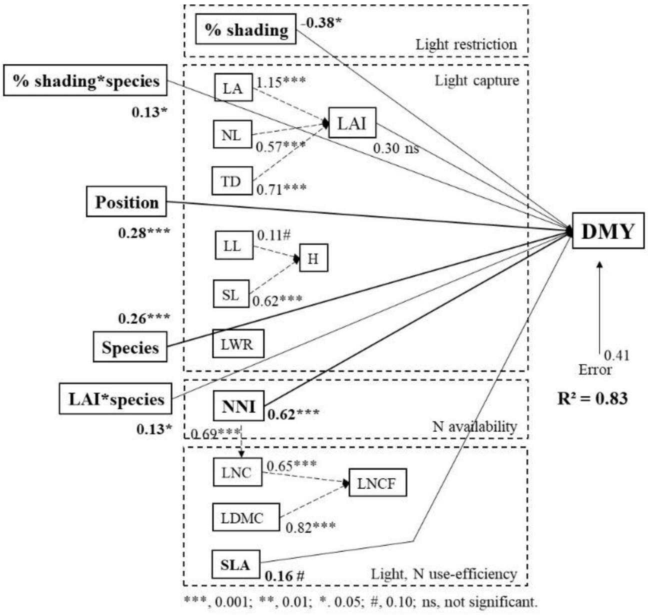

Figure 1. Path analysis model illustrating relations between shoot traits, species, position between tree rows, nitrogen nutrition index (NNI) and shading with dry matter yield (DMY). Significant variables with path coefficients higher than 0.10 are in bold. Arrows for DMY were included for variables selected in stepAIC procedure. LA, leaf area; NL, number of leaves; TD, tiller density; LL, leaf length; SL, sheath length; LWR, leaf weight ratio; LNC, leaf nitrogen content; LDMC, leaf dry matter content; SLA, specific leaf area; LAI, leaf area index; H, canopy height; LNCF, leaf N content per unit of fresh matter.

Some traits had different impact on DMY depending on species, as indicated by significant interactions (Figure 1). The most important interactions were between LAI × species and shading × species. An increase in LAI was associated with an increase in DMY for Mm (+8.6 g DM m^{-2} for each unit of LAI) and Ub (+3.4 g DM m^{-2} for each unit of LAI). LAI is a vital growth parameter for yield determination because determines light capture by the crop. However, for some species (Ha, Ac and Cs), the relationship between LAI with DMY response was negative, with the most significant ($P < 0.05$) negative slope for Ha (-11.3 g DM m^{-2} of decrease in DMY for each unit of increase in LAI). Plasticity for certain traits, particularly for morphological features optimizing light capture, can be high, resulting in a great impact on forage production, particularly for under-story C_4 plants. However, the magnitude of this impact depends on species strategies to cope with multiple stresses (Valladares and Niinemets 2008). DMY decreased with increasing shading for all species except Ub, whose slope (-6.9 g DM m^{-2} per 1% increase in shading) was not significant different from zero ($P = 0.16$). For the other species, the reduction in DMY with increasing in shading ranged from -10.1 (Cs) to -12.5 g DM m^{-2} (Ac) per % of shading.

Conclusions

In conclusion, pathways related to light restriction (i.e., shading level), light capture (LAI via mainly TD and SL), N availability (i.e., NNI) and light and N-use efficiency (via SLA) were associated with DMY response to the factors studied, explaining more than 80% of the variability in DMY. Other species characteristics (e.g., belowground traits) and factors associated to trees presence (e.g., competition for water or nutrients between trees and under-story C_4 plants), not studied by us, may also be important in modulating the biomass productivity of tropical forage species, since species and position factors were also selected in our model with significant path coefficients.

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