

Morphogenesis, biomass and nutritive value of *Panicum maximum* under different shade levels and fertilizer nitrogen rates

D. S. C. Paciullo*, C. A. M. Gomide*, C. R. T. Castro*, R. M. Maurício†, P. B. Fernandes* and M. J. F. Morenz*

*Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, †Federal University of São João Del Rei, São João Del Rei, Minas Gerais, Brazil

Abstract

Panicum maximum is important for cattle production in tropical regions, and it responds well to nitrogen (N) fertilization. Many cultivars have exhibited potential for use in silvopastoral systems, although there is limited information on how different levels of N can influence plant growth and nutritive value under shading. Morphogenetic and structural traits, biomass production and nutritive value of two *P. maximum* cultivars (Tanzânia and Massai) were investigated under three shading levels (0, 37 and 58%) and four N rates (0, 50, 100 and 150 mg N dm⁻³). Plants were grown in pots under shade. Leaf and stem elongation rate and leaf blade length increased with shading and in response to N. Tiller density was reduced with shading, but showed a linear increase fashion with N under full sun and moderate shading. Under intense shading, the tillering showed a quadratic response to N. Plant biomass production increased linearly with N under full sun and moderate shade, but it increased quadratically under intense shade. Crude protein (CP) content increased with shade and N and was greater for Tanzânia than Massai under shade. Contents of NDF and ADF were greater for Massai than Tanzânia. Acid detergent insoluble protein was reduced in Tanzânia under shade, but it increased for Massai. It is suggested that the positive shading effect on CP content may not result in increased nutritional value. Although N fertilization was important for increasing biomass production under full sun and moderate shade, it should be avoided under intense shading.

Keywords: chemical composition, fertilization, forage production, silvopastoral systems, shading, tiller population density

Introduction

Silvopastoral systems can contribute to the sustainability of animal production systems. Attributes of silvopasture include the potential to improve soil fertility and forage quality, increase animal production and thermal comfort, help in the restoration of degraded areas, contribute to carbon sequestration and improved biodiversity and provide income diversification for producers (Murgueitio *et al.*, 2011; Abraham *et al.*, 2014; Paciullo *et al.*, 2014; López-Carrasco *et al.*, 2015). However, the pasture production potential of silvopastures can sometimes be limited by the effect of shade levels imposed by the presence of trees.

Competition for light between trees and herbage components is an important consideration in silvopastoral system management (Neel *et al.*, 2008, 2016; Santiago-Hernández *et al.*, 2016). Shading of more than 50% of the incident radiation can affect the biomass production of tropical forage grasses (Guenni *et al.*, 2008; Abraham *et al.*, 2014), because of the reduced photosynthetic rates in C₄ grasses (Santiago-Hernández *et al.*, 2016). However, under moderate shading, herbage production may be similar or even greater than that observed in open pastures (Kyriazopoulos *et al.*, 2012; Paciullo *et al.*, 2014). Grass tolerance to shade depends on a plant's ability to adapt, morphologically and physiologically, to a given irradiance level (Dias-Filho, 2000). Under moderate shade, grasses can continue to grow at levels that are considered to be satisfactory, with increases in the ratio of above-ground biomass to root biomass (Paciullo *et al.*, 2010), the specific leaf area (Abraham *et al.*, 2014), leaf elongation rate and leaf blade length (Fernández *et al.*, 2002); although with a reduction in the

Correspondence to: D. S. C. Paciullo, Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil.
E-mail: domingos.paciullo@embrapa.br

Received 9 December 2015; revised 9 August 2016

tillering rate but an increased tiller weight (Paciullo *et al.*, 2011; Abraham *et al.*, 2014).

Another aspect to consider in relation to forage production, independent of the adopted system, is that tropical grass growth is severely limited by nitrogen (N) deficiency (Silveira *et al.*, 2015). The evidence shows there are benefits from moderate shading in grass that grows in naturally low-nitrogen soils (Wilson, 1996). One explanation is that the soil moisture content is reduced more slowly under shade than in full sun conditions, which is favourable for microbial activity in litter as well as mineralization, thereby improving nitrogen availability in the soil (Wilson, 1996).

However, for most grasses that demand high soil fertility, it is possible that the nutrient content, especially N, is insufficient in shaded environments to meet the requirements of these grasses (Andrade *et al.*, 2001; Bernardino *et al.*, 2011; Pandey *et al.*, 2011). Tree species, such as eucalyptus and pines used for timber production in silvopastoral systems, are effective at nutrient extraction (Chang *et al.*, 2002; Leite *et al.*, 2010). Thus, competition between trees and grasses is likely to reduce N availability for forage growth. Andrade *et al.* (2001) concluded that low N availability limited the growth of *Panicum maximum* cv. Tanzania when cultivated in a silvopastoral system with *Eucalyptus urophylla*. In addition, N fertilization improved the herbage dry-matter (DM) accumulation, even under shaded conditions. In the case of understorey forages, it has been shown that lower light availability may affect the efficiency of herbage responses to N applications, and this also depends on the availability of N in the soil and in grass species (Guenni *et al.*, 2008; Paciullo *et al.*, 2011; Pandey *et al.*, 2011).

Panicum maximum is one of the most important species for cattle production in tropical and subtropical regions, and it responds well to N fertilization (Freitas *et al.*, 2012). Many *P. maximum* cultivars show medium levels of tolerance to shade (Santiago-Hernández *et al.*, 2016), which indicates they have potential for use in silvopastoral systems. The cultivars Tanzania and Massai have been released from the *P. maximum* breeding programme at EMBRAPA, and because of their advantages over older cultivars they are currently among the most planted *P. maximum* cultivars in Brazil. They have shown favourable characteristics for the intensification of pasture production because of their high dry-matter yield and forage nutritive value, but they also have high demands for soil fertility, especially in terms of nitrogen requirements.

To implement successful silvopastoral systems with these cultivars, it will be necessary to quantify their morphological behaviour, forage production and

nutritive value under different conditions of shading and different levels of N supply.

The hypothesis tested in this experiment was that light intensity affects responses to N applications in forage grasses throughout the re-growth period and that this has implications for the growth pattern and chemical composition of plants. The objective was to evaluate the morphogenetic traits, biomass production and nutritive value of the *P. maximum* cultivars Tanzania and Massai when grown under three levels of shading and four levels of fertilizer N addition.

Materials and methods

This experiment was performed at the EMBRAPA Dairy Cattle Research Center in Juiz de Fora, Minas Gerais, Brazil (21°41'20S, 43°20'W; altitude 678 m above sea level). The regional climate here according to Köppen classification is Cwa (mesothermal). The average monthly rainfall is 60 mm, and the mean air temperature is 17°C from April to September, and the corresponding mean values for October–March are 230 mm and 24°C.

Experimental procedures

The experimental design was completely randomized within a factorial scheme, with three replicates that consisted of two guinea grass (*P. maximum* Jacq) cultivars (Tanzânia and Massai) that were subjected to three levels of artificial shading (0, 37, and 58%) and four rates of fertilizer supplied to soil in pots at 0, 50, 100 and 150 mg N dm⁻³ of soil.

Seeds of cv. Tanzânia and cv. Massai were planted in plastic pots containing a commercial *Pinus* spp. bark substrate. After 20 d of growth, the seedlings were transplanted to tubes, each of which contained 35 cm³ of the same commercial substrate. After 40 d, three seedlings from each genotype were transplanted into 72 plastic pots (36 for each cultivar) containing 5.0 kg of a mixture of soil (Red-Yellow Latosol) and sand at a 4:1 ratio. Limestone was added at amounts equivalent to 2000 kg ha⁻¹. The substrate/soil had the following chemical composition: phosphorus (Mehlich-1), 4.4 mg dm⁻³; potassium, 47 mg dm⁻³; calcium, 0.8 cmolc dm⁻³; magnesium, 0.3 cmolc dm⁻³; H+Al, 1.82 cmolc dm⁻³; and base saturation, 40%, and the pH (H₂O) was equivalent to 6.3.

During the experiment, there were three harvests. These were taken at the end of the following growth periods: 32 d post transplanting, on 9 January 2009; 41 d post first harvest, on 19 February 2009; and 34 d post second harvest, on 25 March 2009.

The experiment was conducted outdoors. Shading was achieved with polypropylene shading cloth of

different meshes, and the cloth was fixed at a 2 m height above the workbenches. Shading cloth was also placed on the sides of the workbenches to prevent the influence of morning and afternoon sunlight incidence. The shading percentages were calculated by measuring the photosynthetically active radiation (PAR) in full sunlight and under the shade cloth. The measurements were performed during the clear (no shade, no clouds) days, in four dates during experimental period (18 December 2008 and 15 January, 10 February and 14 March 2009), with a linear quantum sensor (Ceptometer LP-80; Decagon Devices, Inc., Pullman, WA, USA). The average maximum incident PAR was $2400 \mu\text{mol m}^{-2} \text{s}^{-1}$ for full sun, $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ for moderate shade (37%) and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for intense shade (58%), which occurred at approximately 12:00 to 14:00 h (Figure 1).

Nitrogen (0, 50, 100 and 150 mg dm^{-3} of soil, equivalent to 0, 100, 200 and 300 kg ha^{-1}) was applied to the soil surface of the pots as water-diluted urea at a rate of 50 mL per pot, per application. At the beginning of each re-growth period, potassium (K_2O) and phosphorus (P_2O_5) were applied at 50 mg dm^{-3} (equivalent to 100 kg ha^{-1}) after being mixed with N in a single dose. During the experimental period, irrigation was applied daily to maintain the soil moisture near field capacity.

Measurements

The plant morphogenesis and structural traits were evaluated during each period. The leaf blades and the stems of two tillers in each pot were measured every week. The full length of the leaf blade was measured up to the ligule exposure. The length of the emerging blades was measured from the tip to the ligule of the last expanded leaf, up to the point where its ligule membrane was visible. The date when a leaf appeared was defined as the day when its ligule was first

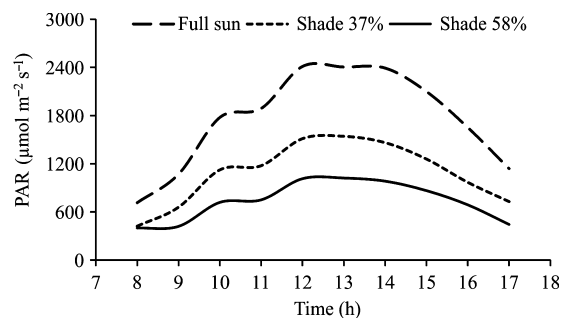


Figure 1 Diurnal patterns of photosynthetic active radiation (PAR) according to shade levels.

observed, and the date of its death was defined as the day when all of its tissue was senescent (Paciullo *et al.*, 2011). The length of each stem was measured at the beginning and end of each evaluation period as the distance from the soil to the ligule of the youngest adult leaf. These data were used to estimate the leaf appearance rate and the leaf and stem elongation rates for each tiller. The total number of leaves per tiller was also determined. The leaf lifespan was estimated using the values for the leaf appearance rate and the number of leaves per tiller (Chapman and Lemaire, 1993). The leaf and stem elongation rates were obtained by dividing the difference between the final and initial total lengths of the leaf blades or stems by the number of days in the growth period. The leaf appearance rate was calculated by dividing the number of fully expanded leaves (with exposed ligules) that appeared per tiller by the number of days in the growth period. The number of tillers per pot was counted at the end of each growth period, before the harvest.

At the end of each growth period, all the plants were cut at 5 cm above the ground surface. The harvested material was separated into green leaf blades, stems and dead biomass. Each component was dried separately in a forced-draught oven at 55°C until constant weight, and then, the forage mass values were measured. Dry matter (DM) was obtained after oven drying at 105°C and ashing at 600°C for 2 h.

For the nutritive value determinations, leaf samples were ground in a Wiley mill equipped with a 1-mm mesh screen, and they were then kept in labelled plastic containers for laboratory analyses. The following methods were used for chemical analyses: N contents (DM basis) were analysed according to the Kjeldahl procedure described by the Association of Official Analytical Chemists (Association of Official Analytical Chemists, 1990) and crude protein (CP) was calculated as $\text{N} \times 6.25$. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) on a DM basis were assayed using the procedure described by van Soest *et al.* (1991) without using α -amylase. The neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP), both presented as a CP percentage, were assayed according to Licitra *et al.* (1996). Because of the low amounts of herbage biomass harvested under the nil-N treatment, there were insufficient samples for analysis. Thus, only the treatments of 50, 100 and 150 mg N dm^{-3} were considered for the nutritive value analyses.

Statistical analysis

The data were analysed using the means from three growth periods. A variance analysis of

non-transformed data was performed using the MIXED procedure in the SAS[®] statistical package (SAS, 2001), by considering the effects of cultivars, N rates and shading levels as fixed effects. The experimental error was considered as a random effect. Whenever the analysis indicated that there was a significant effect for the N rate, the data were subjected to a regression analysis. The estimated averages from the LSMEANS option were compared with Tukey's test. Differences were declared when $P < 0.05$.

Results

Morphogenetic and structural traits

The leaf and stem elongation rates and leaf blade lengths were influenced by the shading percentage, cultivar and N rate ($P < 0.001$), but no significant interactions among these factors were observed (Table 1). These three variables showed a similar pattern in relation to their shading and cultivar, which was characterized by increasing values with shading and higher values for cv. Tanzânia than cv. Massai. Cultivar was the only factor to influence ($P < 0.0001$) the number of green leaves per tiller ($P < 0.0001$), which was greater for Massai than for Tanzânia (Table 1).

In relation to N rate, there was a quadratic effect on the leaf elongation rate ($P = 0.0184$) and leaf blade length ($P = 0.0341$) and a linear positive effect on the stem elongation rate ($P = 0.0017$; Table 2). The phyllochron varied ($P < 0.0001$) only with nitrogen, responding quadratically to N rates (Table 2).

The interactions between shade and N rate and between shade and cultivar influenced the tiller density ($P < 0.0001$; Table 3). The greatest tiller density was presented by cv. Massai cultivar, regardless of the shading percentage. The tiller density was decreased with shading for both cultivars, although they exhibited different magnitudes. The decrease was higher for cv. Massai (43%) than for cv. Tanzânia (30%), when compared under full sun and intense shade. The tiller

density increased linearly with N rate under full sun and moderate shading, but it increased quadratically under intense shade conditions. The tiller density was similar among the three shading percentages under the nil-N treatment, but it was higher under full sun than shade, when increasing doses were applied (Table 3).

Biomass production

The leaf and total biomass varied in their interactions between the N rate and shade percentage ($P = 0.0234$ and $P = 0.0492$ respectively) and the N rate and cultivar ($P = 0.0282$ and $P < 0.0001$ respectively). At the N rates of 0 and 50 mg N dm⁻³, shade had no significant effect on the leaf biomass and total biomass (Table 4). However, the lowest leaf and total forage biomass values were recorded under more intense shading conditions and the highest N rates. The leaf and total forage biomass responded linearly to N under full sun and moderate shading and responded quadratically under more intense shading (Table 4).

The two cultivars were similar in terms of their leaf and total forage biomass values without N fertilization, but cv. Tanzania presented greater leaf and total biomass when N was applied, regardless of the N rate (Table 4).

Chemical composition

The herbage CP content was influenced by interactions between N rate and shading ($P = 0.0015$) and between cultivar and shading ($P < 0.0001$). Fertilization had a positive influence on CP content, with the greatest response to N in treatments under higher shading conditions (Table 5). The shade increased the CP content for both cultivars, although Massai was influenced only under intense shading (54%). Under shading, Tanzânia presented higher CP content than Massai, and in the full sun treatment, there were similar CP contents in the two grasses (Table 5).

The NDIP content was affected by cultivar ($P < 0.0001$), with cv. Massai having a greater value

Table 1 Leaf elongation rate (LER, mm tiller⁻¹ day⁻¹), stem elongation rate (SER, mm tiller⁻¹ day⁻¹), leaf blade length (mm) and number of green leaves (NGL, leaves tiller⁻¹) of *Panicum maximum* cultivars, according to shade levels or cultivar.

Item	Shade (%)			s.e.m.	Cultivar		s.e.m.
	0	37	58		Massai	Tanzânia	
LER	25.1 ^c	29.6 ^b	36.4 ^a	0.12	24.9 ^b	35.9 ^a	0.10
SER	0.06 ^c	0.12 ^b	0.19 ^a	0.008	0.06 ^b	0.19 ^a	0.007
LBL	24.3 ^c	28.7 ^b	33.8 ^a	0.98	23.6 ^b	34.3 ^a	0.80
NGL	3.6 ^a	4.1 ^a	3.8 ^a	0.18	4.7 ^a	3.2 ^b	0.14

Means followed by different letters, lower case letters comparing the effect of shade or cultivar, are different ($P < 0.05$).

s.e.m. = standard error of means.

Table 2 Leaf elongation rate (LER, mm tiller⁻¹ day⁻¹), stem elongation rate (SER, mm tiller⁻¹ day⁻¹), leaf blade length (mm) and phyllochron (days leaf⁻¹) of *Panicum maximum* cultivars, according to nitrogen rate.

Item	Nitrogen rate (mg dm ⁻³)				P-value ¹		s.e.m.
	0	50	100	150	L	Q	
LER	23.9	28.0	34.9	35.0	ns	**	0.13
SER	0.95	1.22	1.46	1.57	*	ns	0.01
LBL	25.7	27.9	32.4	29.7	ns	***	1.14
Phyllochron	11.1	10.3	9.05	9.04	ns	*	0.46

¹Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; ****P* < 0.001; ***P* < 0.01; **P* < 0.05).

s.e.m. = standard error of means.

than cv. Tanzânia (596 vs. 425 g kg⁻¹ of CP; these data not presented in Table 6). The NDIP also varied with the interaction (*P* = 0.0349) between N rate and shading percentage (Table 6). The N effect was observed only under moderate shade (36%), with the lowest value at the lower N rate compared to the other rates. The shading effect varied according to N rate. For the lowest N rate, the greatest NDIP content was observed in full sun condition. For two higher N rates (100 and 150 mg dm⁻³), the NDIP was lower under more intense shade than under full sun and moderate shade.

The ADIP content did not vary with N rates, but there was a significant interaction between cultivar and shade percentage (*P* = 0.0004) for the ADIP (Table 6). Massai presented greater values than Tanzânia, regardless of the shading level. The Massai cultivar showed the largest value under more intense shading, and for Tanzânia, the inverse pattern was

observed, with a higher value recorded for full sun treatments.

The content of NDF was influenced by the shade percentage (*P* = 0.0068), cultivar (*P* < 0.0001) and N rate (*P* = 0.0002), but the interactions between these factors were not significant (*P* > 0.05; Table 7). The NDF content was higher under full sun than under shaded conditions, and higher for cv. Massai than cv. Tanzânia, and also higher under the lowest N rate. The ADF content varied between cultivars (*P* < 0.0001), with a higher value for Massai than for Tanzânia, but did not vary between shade conditions or N rates (Table 7).

Discussion

Although both cultivars present erect growth, the leaves and culms of cv. Tanzânia are naturally larger than the leaves and culms of cv. Massai, which explains the basic morphological differences between the cultivars. The higher leaf and stem elongation rates and leaf length of Tanzânia relative to those of Massai are related to its dimension and also because of its response to shade treatments. The increased leaf elongation rate and leaf length in the shade that was verified in this study is a common plant response to enable increased light capture capacity under low light conditions (Guenni *et al.*, 2008). In addition, shading also stimulated the stem elongation rate for both Tanzânia and Massai, and this is a frequent tendency in plants that are cultivated in the shade (Paciullo *et al.*, 2011). The etiolation of plants subjected to shading is a mechanism through which the vegetation seeks out the light by lifting its leaves along the tiller. In grasses, this mechanism also permits a better distribution of light inside the canopy (Gomide *et al.*, 2011).

Similar to the effects of shading, which strongly stimulated the elongation rates, nitrogen is an

Table 3 Tiller population density (tiller pot⁻¹) of *Panicum maximum* cultivars, according to shade level × nitrogen rate and shade level × cultivar interactions.

Shade (%)	Nitrogen rate (mg dm ⁻³)				P-value ¹		s.e.m.	Cultivar [†]		s.e.m.
	0	50	100	150	L	Q		MA	TA	
0	24.8 ^A	51.2 ^A	66.8 ^A	83.5 ^A	***	ns	1.44	82 ^{Aa}	30 ^{Ab}	1.41
37	28.0 ^A	35.3 ^B	61.6 ^A	65.0 ^B	*	ns	1.44	69 ^{Ba}	25 ^{ABb}	1.41
58	18.7 ^A	29.8 ^B	43.7 ^B	41.2 ^C	ns	*	1.44	47 ^{Ca}	21 ^{Bb}	1.41

Means followed by different letters, upper case letters comparing the effect of shade and lower case letters comparing the effect of cultivar, are different (*P* < 0.05).

s.e.m. = standard error of means.

¹Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; ****P* < 0.001; **P* < 0.05).

[†]MA = cv. Massai; TA = cv. Tanzânia.

Table 4 Leaf and total biomass of *Panicum maximum*, according to shade level × nitrogen rate and cultivar × nitrogen rate interactions.

Item	Nitrogen rate (mg dm ⁻³)				P-value ¹		s.e.m.
	0	50	100	150	L	Q	
<i>Leaf biomass (g DM pot⁻¹)</i>							
Shade							
0	2.1 ^A	6.8 ^A	9.2 ^A	11.9 ^A	**	ns	0.35
37	2.7 ^A	5.9 ^A	9.4 ^A	10.6 ^B	**	ns	0.35
58	2.5 ^A	6.1 ^A	8.1 ^B	8.0 ^C	ns	*	0.35
Cultivar							
Massai	2.5 ^A	5.8 ^B	8.4 ^B	9.5 ^B	*	*	0.29
Tanzânia	2.3 ^A	6.6 ^A	9.6 ^A	11.0 ^A	*	*	0.29
<i>Total biomass (g DM pot⁻¹)</i>							
Shade							
0	2.3 ^A	8.1 ^A	11.4 ^A	13.7 ^A	*	ns	0.44
37	3.1 ^A	8.0 ^A	11.5 ^A	13.6 ^A	**	ns	0.44
58	3.4 ^A	7.5 ^A	9.8 ^B	9.6 ^B	ns	*	0.44
Cultivar							
Massai	2.8 ^A	6.7 ^B	9.9 ^B	11.1 ^B	*	ns	0.36
Tanzânia	3.2 ^A	8.7 ^A	12.0 ^A	15.1 ^A	**	ns	0.36

For each variable, means followed by different letters, comparing the effect of shade or cultivar, are different ($P < 0.05$).

¹Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; ** $P < 0.01$; * $P < 0.05$). s.e.m. = standard error of means.

important factor that influences plant morphogenesis. Increases of 50 and 54% in the leaf and stem elongation rates, respectively, were observed in plants that received the highest N rate compared with nil-N supply. Studies with grasses showed that the meristematic regions make great demands for nitrogen compounds, and their participation in the synthesis of new tissue is effective not only as a source of N but also of carbon skeletons (Gastal and Nelson, 1994).

However, the number of green leaves per tiller was not correlated with the light environment of the

plants or with N rates. In this study, the variation that occurred only between cultivars confirmed that this characteristic is more likely to be determined by the genetics of the given species than the environment in which the plant grows, which is consistent with the findings of Paciullo *et al.* (2011).

Tillering in grasses is a complex phenomenon that is controlled by a number of endogenous and environmental factors (Gautier *et al.*, 1999). The tiller density was reduced in both cultivars with shading, although the decrease for Massai was more intensive than it was for Tanzânia. This result corroborates the results in the literature (Paciullo *et al.*, 2010; Abraham *et al.*, 2014) and reinforces the importance of light in the production of new tillers in grass pastures. The tiller population density also varied with the shading × N rate interaction. Under full sunlight and moderate shading, the response to N was linear; whereas for intense shading, the responses were quadratic, showing that intense shading limited the plant's response to N, in terms of the appearance of new tillers.

A joint analysis of the leaf and stem elongation rate and tiller density data in relation to the N rates indicates that an increase in N availability under shade improved the growth of existing tillers, to the detriment of new tiller production. Forage grasses prioritize the growth of existing tillers, at the expense of new tiller production, to maintain the development of the existing tiller under shaded conditions. Under moderate shade, this mechanism may be sufficient for promoting satisfactory dry-matter accumulation (Sousa *et al.*, 2010). This finding is supported by the morphogenetic changes that occur in response to shade, as observed in this study, besides other characteristics that are influenced by shading, including the increase in the shoot:root ratio and the specific leaf area (Guenni *et al.*, 2008; Paciullo *et al.*, 2010). When taken together, these changes can increase the photosynthesis-to-respiration ratio at the whole-plant level and contribute to the maintenance of a positive carbon balance and the maximization of growth in shade

Table 5 Crude protein content (g kg⁻¹ of DM) in leaves of *Panicum maximum*, according to shade level × nitrogen rate and shade level × cultivar interactions.

Shade (%)	Nitrogen rate (mg dm ⁻³)			s.e.m.	Cultivar		s.e.m.
	50	100	150		Massai	Tanzânia	
0	84 ^{Bb}	104 ^{Ca}	115 ^{Ca}	0.38	96 ^{Ba}	105 ^{Ba}	0.39
37	112 ^{Ab}	120 ^{Bb}	142 ^{Ba}	0.38	109 ^{Bb}	141 ^{Aa}	0.39
58	117 ^{Ac}	143 ^{Ab}	171 ^{Aa}	0.38	129 ^{Ab}	159 ^{Aa}	0.39

Means followed by different letters, (lower case letters comparing the effect of nitrogen rate or cultivar and upper case letters comparing the effect of shade) are different ($P < 0.05$).

s.e.m. = standard error of means.

Shade (%)	Nitrogen rate (mg dm ⁻³)			s.e.m.	Cultivar		s.e.m.
	50	100	150		Massai	Tanzânia	
	NDIP (g kg ⁻¹ of CP)				ADIP (g kg ⁻¹ of CP)		
0	569 ^{Aa}	536 ^{Aa}	535 ^{Aa}	15.9	96 ^{Ba}	59 ^{Ab}	5.7
37	472 ^{Bb}	529 ^{Aa}	517 ^{Aa}	15.9	95 ^{Ba}	51 ^{ABb}	6.9
58	491 ^{Ba}	479 ^{Ba}	470 ^{Ba}	15.9	123 ^{Aa}	45 ^{Bb}	5.4

Means followed by different letters, (lower case letters comparing the effect of N rate or cultivar and upper case letters comparing the effect of shade) are different ($P < 0.05$).

s.e.m. = standard error of means.

Table 7 Neutral detergent fibre (NDF) and acid detergent fibre (ADF) (g kg⁻¹ of DM) in leaves of *Panicum maximum*, according to shade level, cultivar and nitrogen rate.

Item	NDF	ADF
Shade (%)		
0	651.8 ^a	315.1
37	642.3 ^b	330.1
58	642.1 ^b	330.0
s.e.m.	2.73	4.42
Cultivar		
Massai	657.7 ^a	347.1 ^a
Tanzânia	633.1 ^b	303.1 ^b
s.e.m.	2.23	3.61
N rate (mg dm ⁻³)		
50	656.0 ^a	322.8
100	643.4 ^b	333.0
150	636.7 ^c	319.4
s.e.m.	2.73	4.42

Means followed by different letters, in column, comparing the effect of shade, cultivar or nitrogen rate, are different ($P < 0.05$).

s.e.m. = standard error of means.

(Kitajima, 1994). In addition, the possibility of increasing the growth of existing tillers under moderate shading can be associated with high N uptake by moderately shaded stands. The higher N concentration in shaded leaves contributes to improvements in the photosynthetic light response, resulting in higher radiation-use efficiency in unfertilized plants or in plants that are receiving a low N supply (Cruz, 1997). This mechanism may explain the similar biomass production that occurs among light environments, including intense shading, when no N or 50 mg dm⁻³ of N was applied.

The leaf and total biomasses values increased linearly with N rate in both full sun and under moderate

Table 6 Neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) in leaves of *Panicum maximum*, according to shade level × nitrogen rate and shade level × cultivar interactions.

shading, but they increased quadratically under intense shading, reflecting the influence of low luminosity on plant responses to N applications. Under moderate shade, some tropical grasses (*Brachiaria decumbens*, *B. brizantha* and *P. maximum*) can continue to grow at levels that are considered to be satisfactory, when compared with full sun condition (Paciullo *et al.*, 2010; Gómez *et al.*, 2013; Santiago-Hernández *et al.*, 2016). Plant acclimation under restricted irradiance implies a substantial carbon investment in leaf area expansion for light capture. This is related to changes in biomass allocation, prioritizing the growth of shoots at the expense of the root system (Guenni *et al.*, 2008; Paciullo *et al.*, 2010), and to increased leaf elongation rate, as observed in present study. Furthermore, under restricted light environments, plants may compensate efficiently for low irradiance by increasing the specific leaf area, minimizing the cost of tissue construction (Gómez *et al.*, 2013). From a physiological perspective, recently expanded leaves are capable of maintaining their functional integrity for photosynthesis so that light-use efficiency is maintained, or even increased, compared with leaves developed under full sunlight (Gómez *et al.*, 2013).

However, the decreasing biomass production under intensive shading, associated with the two highest N rates, reflected the negative effect of severe light restriction on biomass production. Although the N level was the primary limiting factor for growth under highlight conditions, even under low light the plants could not respond to the highest N rate under this insufficient light level. The impact on DM yield of intense shade, which normally blocked more than 50% of the light from full sun exposure, has also been noted by other authors (Durr and Rangel, 2000; Guenni *et al.*, 2008). Barnes *et al.* (2015) found that shade greatly reduced biomass accumulation of a native Australian C₄ species, *Chloris ventricosa*, but had little effect on two native C₃ grasses. Under conditions

of intense and permanent shade, the low photosynthetic capacity significantly decreased the biomass production of tropical forage grasses (Santiago-Hernández *et al.*, 2016), which indicates that excessive shading should be avoided if high biomass production is required.

The increase in biomass production in response to N rates, observed for both cultivars in the present study, is in agreement with previous research with *P. maximum* (Freitas *et al.*, 2012; Cardoso *et al.*, 2016). This result reflected the positive influence of N on the leaf and stem elongation rates and on the tillering of both cultivars. Although the cultivars responded positively to N, the potential for leaf and total biomass production of grasses varied according to N rate. At the lowest N rate, the biomass values of the grasses were similar. However, when N was applied, cv. Tanzânia presented higher values of leaf and total biomass compared with cv. Massai. This pattern indicates the greater potential of Tanzânia relative to that of Massai under N fertilization and suggests that Tanzânia is more suitable than Massai for intensive production systems.

The observed increase in the CP content in response to shade is consistent with the results found in other studies (Neel *et al.*, 2008; Sousa *et al.*, 2010; Paciullo *et al.*, 2014). Several hypotheses have been advanced to explain this effect. In field experiments, Wilson (1996) attributed this phenomenon to increasing soil moisture, which improves organic matter degradation and nitrogen recycling in the soil under shaded conditions. Thus, the high CP contents in the pasture herbage could be associated with an increased flow of nitrogen into the soil, especially when the tree component consists of legumes with potential to contribute to biological N fixation (Xavier *et al.*, 2014). Although this explanation may apply under field conditions for silvopasture systems, in our experiment, water was not a growth-limiting factor. In this context, Sousa *et al.* (2010) discussed another mechanism related to a delay in the ontogenetic development of plants cultivated under more intense shade. In this case, grasses tended to be physiologically younger, which prolongs the juvenile vegetative phase and allows for the maintenance of higher metabolic levels for a longer period of time. Neel *et al.* (2016) showed there was a 4- to 6-d delay for silvopasture to reach an equivalent morphological maturity as swards growing in full sunlight. Furthermore, the increased CP content in shaded plants could be associated with the reduced cell size caused by reduced light. Kephart and Buxton (1993) speculated that the reduced cell size, along with a constant quantity of N per cell, may have a concentrating effect.

Although shading increases the CP content of grasses, N fertilization has potential for a CP

accumulation effect, especially under intense shade. The highest shading level had a negative impact on biomass production, and N assimilation apparently did not decrease relative to biomass production. Dale and Causton (1992) reported higher N accumulation in plants growing under shade and concluded that because N uptake exceeds metabolic requirements, there was an imbalance in the C and N assimilations. From a nutritional perspective, high CP concentration can be advantageous for ruminant nutrition, although there is evidence that a high N leaf concentration as a result of intense shading condition may result in an excessive leaf nitrate concentration, which could in turn reduce the dry-matter intake by ruminants (Neel *et al.*, 2016).

Tanzânia showed higher CP content than cv. Massai, in the shading conditions tested, although no difference was observed in full sun. A previous study showed greater CP content for Tanzânia than Massai (Brâncio *et al.*, 2002). The greater CP content, associated with lower NDF and ADF, indicates superior nutritional value of cv. Tanzânia relative to cv. Massai. This result may seem contradictory when considering the leaf biomass in relation to total biomass at the highest N rate (86 and 71% for cv. Massai and cv. Tanzania respectively) calculated from the values in Table 4. However, it should be emphasized that the analyses of nutritional values were carried out using the leaf fraction, the preferred component selected by grazing animals (Brâncio *et al.*, 2003).

The NDIP did not vary with the N rate, except under moderate shading at which it was lower for the lower fertilizer N rate. There is no obvious biological explanation for this, in view of the absence of an N effect in other light conditions. Regarding the shading level, similar values of NDIP were observed when comparing full sun and moderate shade (except the value obtained at the lowest N rate) and decreased NDIP under intense shade in relation to full sunlight. The lack of difference in the full sun and moderate shading treatments is consistent with Neel *et al.* (2016), who also found no differences in NDIP in open pasture and silvopasture with a maximum reduction of 20% of solar radiation. These results indicate that reductions in NDIP contents are conditioned to intense shading.

The interaction between the shade level and the cultivar with regard to ADIP was due to the cv. Massai showing greater values with intense shading, while cv. Tanzânia presented decreasing ADIP contents in response to shade. The result observed for Tanzânia is in agreement with Neel *et al.* (2016) who also found lower ADIP in silvopasture compared with open pasture (i.e. a non-shaded environment). In this context, the increased CP content of Tanzânia in shaded

compared with non-shaded conditions could represent an advantage in nutritional value. For cv. Massai, there was a tendency for both ADIP and CP contents to have greatest values under intense shade. The ADIP is the N fraction which accounts for the unavailable protein contained in the cell walls that is undegraded in the rumen and is non-digestible in ruminant intestines (Sniffen *et al.*, 1992), and it could compromise the nutritive value. This result suggests that the positive shading effect on the CP content may not necessarily result in an increased forage nutritional value.

The NDF showed only a slight decrease, and the ADF contents were similar among shading levels. In general, the NDF and ADF concentrations of most grass species are either increased or unaffected by shade (Sousa *et al.*, 2010; Paciullo *et al.*, 2014; Neel *et al.*, 2016). The slight decrease in NDF recorded in this study under shade could be associated with the thinner cell walls of the sclerenchyma in plants that are cultivated under shade than under full sun conditions (Deinum *et al.*, 1996). This explanation is supported by results from Kephart and Buxton (1993), who found a decreasing tendency in secondary cell wall development shade conditions. Neel *et al.* (2016) also showed a 4- to 6-d delay in morphological maturation within silvopastoral swards compared to open pasture.

The cv. Massai had greater NDF and ADF contents than Tanzânia, which is in agreement with previous research (Brâncio *et al.*, 2002; Euclides *et al.*, 2008). The higher fibre components in Massai could be associated with its anatomical traits. Gomes *et al.* (2011) observed a greater area of sclerenchymatic tissue in Massai leaves than in Tanzânia. This in turn results in great leaf cell wall content due to great proportion of cell wall in sclerenchyma tissue. Furthermore, previous studies have shown that the arrangement of sclerenchyma cells between epidermal and bundle sheath cells (girder structure) results in a strong structure of lignified tissues that contribute to leaf rigidity. This may reduce herbage DM digestibility and animal productivity of Massai pastures compared with other *Panicum* cultivars (Brâncio *et al.*, 2002; Euclides *et al.*, 2008; Gomes *et al.*, 2011).

Implications for field conditions

This study confirmed our hypothesis that the interaction between shade and N fertilization strongly affects biomass production and the nutritional value of *Panicum* cultivars. Nitrogen fertilization was shown to be an important strategy for increasing biomass production and herbage CP content under both full sun and moderate shading conditions, and it should be

considered for use in silvopastoral systems. Although the low N rate increased the biomass production at 58% shade, the application of high N rates to swards that are managed under intense shading should be avoided because of their low fertilization efficiency. Moreover, the increase in the non-degradable protein fraction (ADIP) of the Massai cultivar under intense shading suggests that the positive shading effect on the crude protein content may not necessarily result in an increased forage nutritional value.

Regardless of the cultivar, high levels of forage production should not be expected from pasture swards that are subjected to intense shading, because the morphogenetic changes that were observed under this condition are apparently insufficient to allow high rates of herbage production. Moreover, a marked reduction in the tiller population as observed in this study may compromise the long-term survival of these perennial forage grasses, with possible negative effects on sward persistence under grazing.

Of the two *P. maximum* cultivars studied here, Tanzânia showed the higher productive and nutritive potential, especially under full sun or moderate shade in association with a high N supply, which suggests that Tanzânia is more appropriate than Massai for use in intensive production systems. However, this study was performed under controlled artificial shade in which moisture was not a limiting factor, and there were no other plants such as trees to compete for moisture and nutrients. More studies are needed to confirm these results in silvopastoral systems before recommendations can be made under field conditions.

Acknowledgments

The authors thank the Minas Gerais Research Foundation (FAPEMIG) for funding of this research. We also thank the following people for their valuable contribution to this research: Dr. Marco Antônio Sundfeld da Gama for suggestions to improve the manuscript and to the undergraduate students Igor Costa and Aline Lima for their contributions during the field trial setup.

References

- ABRAHAM E.M., KYRIAZOPOULOS A.P., PARISSI Z.M., KOSTOPOULOU P., KARATASSIOU M., ANJALANIDOU K. and KATSOUTA C. (2014) Growth, dry matter production, phenotypic plasticity, and nutritive value of three natural populations of *Dactylis glomerata* L. under various shading treatments. *Agroforestry Systems*, **88**, 287–299.
- ANDRADE C.M.S., GARCIA R. and COUTO L. (2001) Fatores limitantes ao crescimento do capim-tanzânia em

- um sistema agrossilvopastoril com eucalipto, na região dos cerrados de Minas Gerais. (Factors limiting the growth of *Panicum maximum* cv. Tanzânia-1 in an agrossilvopastoral system with eucalypt in the Cerrados of Minas Gerais). *Revista Brasileira de Zootecnia*, **30**, 1178–1185.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. (1990) *Official methods of analysis*, 15th edn, p. 1298. Washington DC: AOAC.
- BARNES P., WILSON B.R., REID N., BAYERLEIN L., KOEN T.B. and OLUPOT G. (2015) Examining the impact of shade on above-ground biomass and normalized difference vegetation index of C₃ and C₄ grass species in North-Western NSW, Australia. *Grass and Forage Science*, **70**, 324–334.
- BERNARDINO F.S., TONUCCI R.G. and GARCIA R. (2011) Produção de forragem e desempenho de novilhos de corte em um sistema silvopastoril: efeito de doses de nitrogênio e oferta de forragem. (Forage yield and performance of beef steers in a silvopastoral system: effects of forage offers and nitrogen fertilization). *Revista Brasileira de Zootecnia*, **40**, 1412–1419.
- BRÂNCIO P.A., NASCIMENTO JR. D., EUCLIDES V.P., REGAZZI A., ALMEIDA R.G., FONSECA D.M. and BARBOSA R.A. (2002) Avaliação de três cultivares de *Panicum maximum* Jacq. sob pastejo. Composição química e digestibilidade da forragem. (Evaluation of three cultivars of *Panicum maximum* Jacq. under grazing. Chemical composition and *in vitro* organic matter digestibility). *Revista Brasileira de Zootecnia*, **31**, 1605–1613.
- BRÂNCIO P.A., EUCLIDES V.P.B., NASCIMENTO JR. D., FONSECA D.M., ALMEIDA R.G., MACEDO M.C.M. and BARBOSA R.A. (2003) Avaliação de três cultivares de *Panicum maximum* Jacq. sob pastejo: disponibilidade de forragem, altura do resíduo pós-pastejo e participação de folhas, colmos e material morto. (Evaluation of three cultivars of *Panicum maximum* Jacq. under grazing: forage availability, post grazed stubble height and participation of leaves, stems, and dead material). *Revista Brasileira de Zootecnia*, **32**, 55–63.
- CARDOSO S., VOLPE E. and MACEDO M.C.M. (2016) Effect of nitrogen and lime on Massai grass subjected to intensive cutting. *Pesquisa Agropecuária Tropical*, **46**, 19–27.
- CHANG S.X., AMATYA G., BEARE M.H. and MEAD D.J. (2002) Soil properties under a *Pinus radiata* – ryegrass silvopastoral system in New Zealand. Part I. Soil N and moisture availability. *Agroforestry Systems*, **54**, 137–147.
- CHAPMAN D.F. and LEMAIRE G. (1993) Morphogenetic and structural determinants of plant regrowth after defoliation. In: *Proceedings of the XVII International Grassland Congress, Palmerston North, Hamilton, Lincoln and Rockhampton (New Zealand and Australia)*, 8–21 February 1993, pp. 95–104.
- CRUZ P. (1997) Effect of shade on the growth and mineral nutrition of a C₄ perennial grass under field conditions. *Plant and Soil*, **188**, 227–237.
- DALE M.P. and CAUSTON D.R. (1992) The ecophysiology of *Veronica chamaedrys*, *V. montana* and *V. officinalis*. IV. Effects of shading on nutrient allocations – a field experiment. *Journal of Ecology*, **80**, 517–526.
- DEINUM B., SULASTRI R.D., ZEINAB M.H.J. and MAASSEN A. (1996) Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and *Panicum maximum* var. Trichoglume). *Netherlands Journal of Agricultural Science*, **44**, 111–124.
- DIAS-FILHO M. (2000) Growth and biomass allocation of the C₄ grasses *Brachiaria brizantha* and *B. humidicola* under shade. *Pesquisa Agropecuária Brasileira*, **35**, 2335–2341.
- DURR P.A. and RANGEL J. (2000) The response of *Panicum maximum* to a simulated subcanopy environment. I. Soil × shade interaction. *Tropical Grasslands*, **34**, 110–117.
- EUCLIDES V.P., MACEDO M.C.M., ZIMMER A.H., JANK L. and OLIVEIRA M.P. (2008) Avaliação dos capins Mombaça e Massai sob pastejo. (Evaluation of *Panicum maximum* cvs Mombaça and Massai under grazing). *Revista Brasileira de Zootecnia*, **37**, 18–26.
- FERNANDÉZ M.E., GYENGE J.E., SALDA DALLA G. and SCHLICHTER T.M. (2002) Silvopastoral systems in northwestern Patagonia. I: growth and photosynthesis of *Stipa speciosa* under different levels of *Pinus ponderosa* cover. *Agroforestry Systems*, **55**, 27–35.
- FREITAS F.P., FONSECA D.M., BRAZ T.G.S., MARTUSCELLO J.A. and SANTOS M.E.R. (2012) Forage yield and nutritive value of Tanzania grass under nitrogen supplies and plant densities. *Revista Brasileira de Zootecnia*, **41**, 864–872.
- GASTAL F. and NELSON C.J. (1994) Nitrogen use within the growing leaf blade of tall fescue. *Plant Physiology*, **105**, 191–197.
- GAUTIER H., VARLET-GRANCHER C. and HAZARD L. (1999) Tillering responses to the light environment and to the defoliation in populations of perennial ryegrass (*Lolium perenne* L.) selected for contrasting leaf length. *Annals of Botany*, **83**, 423–429.
- GOMES R.A., LEMPP B., JANK L., CARPEJANI G.C. and MORAIS M.G. (2011) Características anatômicas e morfofisiológicas de lâminas foliares de genótipos de *Panicum maximum*. (Anatomical and morphophysiological leaf blade traits of *Panicum maximum* genotypes). *Pesquisa Agropecuária Brasileira*, **46**, 205–211.
- GÓMEZ S., GUENNI O. and BRAVO DE GUENNI L. (2013) Growth, leaf photosynthesis and canopy light use efficiency under differing irradiance and soil N supplies in the forage grass *Brachiaria decumbens* Stapf. *Grass and Forage Science*, **68**, 395–407.
- GOMIDE C.A.M., PACIULLO D.S.C., COSTA I.A., LIMA A.M., CASTRO C.R.T. and LÉDO F.J.S. (2011) Morphogenesis of dwarf elephant grass clones in response to intensity and frequency of defoliation in dry and rainy seasons. *Revista Brasileira de Zootecnia*, **40**, 1445–1451.
- GUENNI O., SEITER S. and FIGUEROA R. (2008) Growth responses of three *Brachiaria* species to light intensity and nitrogen supply. *Tropical Grasslands*, **42**, 75–87.

- KEPHART K.D. and BUXTON D.R. (1993) Forage quality responses of C₃ and C₄ perennial grasses to shade. *Crop Science*, **33**, 831–837.
- KITAJIMA K. (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia*, **98**, 419–428.
- KYRIAZOPOULOS A.P., ABRAHAM E.M., PARISSI Z.M., KOUKOURA Z. and NASTIS A.S. (2012) Forage production and nutritive value of *Dactylis glomerata* and *Trifolium subterraneum* mixtures under different shading treatments. *Grass and Forage Science*, **68**, 72–82.
- LEITE F.P., SILVA I.R., NOVAIS R.F., BARROS N.F. and NEVES J.C.L. (2010) Alterations of soil chemical properties by *Eucalyptus* cultivation in five regions in the Rio Doce Valley. *Revista Brasileira de Ciência do Solo*, **34**, 821–831.
- LICITRA G., HERNANDEZ T.M. and VAN SOEST P. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, **57**, 347–358.
- LÓPEZ-CARRASCO C., LÓPEZ-SÁNCHEZ A., SAN MIGUEL A. and ROIG S. (2015) The effect of tree cover on the biomass and diversity of the herbaceous layer in a Mediterranean dehesa. *Grass and Forage Science*, **70**, 639–650.
- MURGUEITIO E., CALLE Z., URIBE F., CALLE A. and SOLORIO B. (2011) Native trees and shrubs for the productive rehabilitation of tropical cattle ranching lands. *Forest Ecology and Management*, **261**, 1654–1663.
- NEEL J.P.S., FELDHAKI C.M. and BELESKY D.P. (2008) Influence of solar radiation on the productivity and nutritive value of herbage of cool-season species of an understorey sward in a mature conifer woodland. *Grass and Forage Science*, **63**, 38–47.
- NEEL J.P.S., FELTON E.E.D., SINGH S., SEXTONE A.J. and BELESKY D.P. (2016) Open pasture, silvopasture and sward herbage maturity effects on nutritive value and fermentation characteristics of cool-season pasture. *Grass and Forage Science*, **71**, 259–269.
- PACULLO D.S.C., CASTRO C.R.T., GOMIDE C.A.M., FERNANDES P.B., DUARTE W.S.R., MÜLLER M.D. and ROSSIELLO R.O.P. (2010) Soil bulk density and biomass partitioning of *Brachiaria decumbens* in a silvopastoral system. *Scientia Agricola*, **67**, 401–407.
- PACULLO D.S.C., FERNANDES P.B., GOMIDE C.A.M., CASTRO C.R.T., SOUZA SOBRINHO F.S. and CARVALHO C.A.B. (2011) The growth dynamics in *Brachiaria* species according to nitrogen dose and shade. *Revista Brasileira de Zootecnia*, **40**, 270–276.
- PACULLO D.S.C., PIRES M.F.A., AROEIRA L.J.M., MORENZ M.J.F., MAURICIO R.M. and GOMIDE C.A.M. (2014) Sward characteristics and performance of dairy cows in organic grass-legume pastures shaded by tropical trees. *Animal*, **8**, 1264–1271.
- PANDEY C.B., VERMA S.K. and DAGAR R.C. (2011) Forage production and nitrogen nutrition in three grasses under coconut tree shades in the humid-tropics. *Agroforestry Systems*, **83**, 1–12.
- SANTIAGO-HERNÁNDEZ F., LÓPEZ-ORTIZ S., ÁVILA-RESÉNDIZ C., JARILLO-RODRÍGUEZ J., PÉREZ-HERNÁNDEZ P. and GUERRERO-RODRÍGUEZ J.D. (2016) Physiological and production responses of four grasses from the genera *Urochloa* and *Megathyrsus* to shade from *Melia azedarach* L. *Agroforestry Systems*, **90**, 339–349.
- SAS. (2001) *SAS/STAT Users guide, version 8.1*, vol. 1, 943 pp. Cary, NC: SAS Institute.
- SILVEIRA M.L., VENDRAMINI J.M.B., SELLERS B., MMONTEIRO F.A., ARTUR A.G. and DDUPAS E. (2015) Bahiagrass response and N loss from selected N fertilizer sources. *Grass and Forage Science*, **70**, 154–160.
- SNIFFEN C.J., O'CONNOR J.D., VAN SOEST P.J., FOX D.G. and RUSSEL J.B. (1992) A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. *Journal of Animal Science*, **70**, 3562–3577.
- VAN SOEST P.J., ROBERTSON P.J. and LEWIS J.B. (1991) A method for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, **74**, 3583–3597.
- SOUSA L.F., MAURÍCIO R.M., MOREIRA G.R., GONÇALVES L.C., BORGES I. and PEREIRA L.G.R. (2010) Nutritional evaluation of 'Braquiarião' grass in association with 'Aroeira' trees in a silvopastoral system. *Agroforestry Systems*, **79**, 179–189.
- WILSON J.R. (1996) Shade-stimulated growth and nitrogen uptake by pasture grasses in a subtropical environment. *Australian Journal of Agriculture Research*, **47**, 1075–1093.
- XAVIER D.F., LÉDO F.J.S., PACULLO D.S.C., URQUIAGA S., ALVES B.J.R. and BODDEY R.M. (2014) Nitrogen cycling in a *Brachiaria*-based silvopastoral system in the Atlantic forest region of Minas Gerais, Brazil. *Nutrient Cycling in Agroecosystems*, **99**, 45–62.