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Recommended fertilization and timing of nitrogen fertilization influences the morphogenesis, structural characteristics, and production efficiency of Mombaça grass

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ABSTRACT. This study aimed to evaluate whether different fertilization strategies influence the morphogenic, structural, and production characteristics of *Megathyrsus maximus* cv. Mombaça. The experimental design adopted was a randomized block design with a 4 × 3 factorial scheme having five replications. The treatments were as per the following fertilization recommendations: 5th Approach Guide (5AP), Pará Guide (PG), Nutrient Replacement (RP), and the Michaelis and Menten adapted model (MM), applied for three days after application of the nitrogen (N) source following defoliation (days 0, 3, and 6). The interaction between factors affected the following variables: leaf appearance rate (LAR), leaf elongation rate (LER), and leaf senescence rate (LSR). The highest stem elongation rate (SER) was observed for the 5AP recommendation treatment with N application on day 0 (0.113 cm). The highest leaf area index (LAI; 5.33) and tiller population density (TPD; 421.5 tillers m^{-2}) were observed for the 5AP recommendation. The 5AP recommendation with N application on day 0 and on the sixth day after defoliation, and the MM recommendation with fertilization on day 0 showed the best LAR and LER, as well as a greater number of cycles and greater absolute values of leaf blade dry mass.

Keywords: fertilization; pasture management; *Megathyrsus maximus*; urea.

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Introduction

One of the main reasons for the reduction in the productive potential of grasses in the Amazon region is inadequate nutritional management, which makes soil correction and fertilization necessary to meet the plants' nutritional requirements.

There are several recommendations for pasture fertilization, such as the guidance of correctives and fertilizers - the 5th approach of Minas Gerais (Cantarutti, Alvarez, & Ribeiro, 1999) and the liming and fertilization guide of the state of Pará (Teixeira, Oliveira, & Veiga, 2007). Another model proposed by Lana et al. (2015) was evaluated using the Michaelis-Menten enzyme kinetic saturation model, the objective of which is to recommend levels in a more rational way. Another proposal is the replacement of nutrients extracted by plants during the production process (Brasil, Viégas, Silva, & Gato, 1999). These recommendations differ, especially in the amount of nitrogen (N) supplied, thus making it necessary to evaluate the responses of pastures to fertilization based on these recommendations for the region.

Tropical grass pastures intensively managed during the rainy season were fertilized with nitrogen after the animals were removed from the paddocks. However, the soil is highly susceptible to nitrogen losses (Gomide, Paciullo, Morenz, Costa, & Lanzoni, 2019), and alternatives are needed to improve nitrogen utilization by plants. Notably, after defoliation, there is a rapid decline in the amount of soluble carbohydrates in the roots as a consequence of the reduced photosynthetic rate and preferential allocation of carbon to the shoots of the grass, in order to restore the leaf area (Santos et al., 2011). Therefore, these factors should be considered when determining the ideal time for cover fertilization with nitrogen after defoliation. We hypothesized that there may be a relationship between fertilizer recommendations and the day of N application after defoliation, in terms of nitrogen utilization efficiency of the plant. The understanding of this relationship may guide the producers in choosing a fertilization management method that best suits their production system.

Therefore, the purpose of this study was to evaluate whether different fertilization recommendations and days of N fertilization influence the morphogenesis, structural characteristics, and production efficiency of the grass *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombaça.

Material and Methods

The study was conducted from December 20, 2018, to July 20, 2019, in the Forage Crops Sector of the Federal Rural University of Amazônia, Parauapebas Campus, Pará. The experimental area was located at latitude 06º04'16.4" south, longitude 49º08'8.3" west, and an altitude of 270 m. The soil in the area is classified as red-yellow Argisol (Santos et al., 2018). The climate of the region is classified as Aw-Tropical, with a dry season from May to October and a rainy season from November to April, according to Köppen. The average air temperature in the experimental period was 26.74°C (with a maximum of 35.5+0.2°C and a minimum of 20.7+0.2°C) and the cumulative rainfall was 1,486.8 mm. For the chemical and physical characterization of the experimental area, soil analysis was performed at a depth of 0-20 cm (Table 1).

Table 1. Chemical and granulometric characteristics of the soil of the experimental area in the layer 0-20 cm deep.

pH	P(Melich)	K	Ca^{2+}	Mg^{2+}	Al	H+Al	V%	CEC	OM	Clay	Silt	Sand
($CaCl_2$)	mg dm^{-3}		----------------cmol$_c$ dm^{-3}---------------						g dm^{-3}	dag.kg^{-1}		
4.6	0.8	121.3	2.2	0.7	0.2	2.2	59.33	5.41	21.0	310	80	610

Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), hydrogen + aluminum (H + Al), base saturation (V%), cation exchange capacity (CEC), organic matter (OM), calcium chloride ($CaCl_2$). Source: SOLOCRIA *Laboratório Agropecuário Ltda*, Goiânia - GO, 2017.

Sowing of *Megathyrsus maximus* cv. Mombaça was performed in January (2018) in a semi-mechanized manner with a sprayer regulated for application of 15 kg ha^{-1}, using 4 kg of embedded-type seeds with a crop value (CV) of 80%.

The experiment was carried out using a randomized block design, with a 4 × 3 factorial scheme, including four fertilization recommendations (5th Approach - 5AP; Nutrient Replacement - RP; Liming and Fertilization Guide of Pará State - PG; and Michaelis-Menten Model - MM), and there were three days of application of nitrogen fertilizer (N) after defoliation (N application after defoliation - Day 0; N application on the third day after defoliation - Day 3; and N application on the sixth day after defoliation - Day 6), with five replications, totaling 60 plots with an area of 9 m^2 (3 × 3) each, separated by 0.70-m wide corridors.

The recommendations and amounts of fertilizer expected to be used in the study for each fertilization strategy are shown in Table 2.

Table 2. Nitrogen (N), phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) values predicted for each fertilization strategy to be applied over one (1) crop year, with fertilizations occurring in the rainy season, comprising seven (7) cycles.

	Recommendations	Fertilizer (kg ha^{-1} year^{-1})		
		N	P_2O_5	K_2O
5th Approach Guide (5AP)	According to the recommendation for production system of medium technological level (Cantarutti et al., 1999).	300	70	-
Nutrient Replacement (RP)	According to the equation adapted from Brasil et al. (1999), where: "Fertilization = (Requirement + Factor) – supply", with the requirement being calculated according to Castagnara et al. (2014), Sousa et al. (2010) and Freitas et al. (2011); the factor according to Oliveira, Favaretto, Roloff and Fernandes (2010); and the supply according to Braz et al. (2002).	150	16	60
Pará Guide (PG)	According to the recommendation for semi-intensive and intensive rotational grazing systems (Teixeira et al., 2007).	60	80	-
Michaelis - Menten Model (MM)	As described and adapted by Lana et al. (2015), in which, data from the literature (Mello et al., 2008) was used to apply to the enzyme kinetic saturation model of Michaelis and Menten (1913), using the linear equation of Lineweaver-Burk (1934), obtaining the kinetic constants Ks (amounts of N and P needed to reach half of the theoretical maximum growth rate, with no application of K_2O due to availability in the soil).	110	10	-

Fertilization with P (single superphosphate, 17% P_2O_5) and K (potassium chloride, 60% K_2O) was performed at the beginning of the rainy season, after the pasture was uniform. Urea (45% N) was used for nitrogen fertilization in split doses for seven applications (expected number of production cycles). The defoliation time was determined by considering the height for harvest and residue of 70 and 30 cm, respectively, according to Santos et al. (2021).

The marked tiller technique was used (Davies, 1993). Five tillers were identified per experimental unit five days after the uniformization cut, totaling 25 (twenty-five) tillers per treatment. The leaves of each tiller were evaluated weekly and marked after cutting (until the time of a new cut). Leaf blade lengths were measured and the leaves were classified as either expanding, expanded, senescent, or dead leaves. The stem length was measured from the ground to the ligule of the youngest fully expanded leaf.

The following variables were calculated and determined according to the methodology described by Alexandrino, Candido and Gomide (2011): leaf appearance rate (LAR, cm of leaf tiller^{-1} day^{-1}), leaf elongation rate (LER, cm of leaf tiller^{-1} day^{-1}), stem elongation rate (SER, cm of pseudo stem tiller^{-1} day^{-1}), leaf senescence rate (LSR, cm of leaf tiller^{-1} day^{-1}), tiller population density (TPD, tillers m^{-2}) and leaf area index (LAI, m^2 m^{-2}).

The height of the pasture was continuously monitored; when it reached 70 cm, the forage was harvested using a rectangular frame of 0.6 m^2, leaving 30 cm of residue. The samples were weighed on a semi-analytical scale; thereafter, an aliquot was taken to determine the dry mass and another to separate the morphological components (i.e., leaf (leaf blades), pseudo-stem, and dead plant material). The samples were dried in a forced ventilation oven at 55°C for 72h to obtain the total forage dry mass (TFDM), leaf blade dry mass (LBDM), stem dry mass (SDM), and dead material dry mass (DMDM) according to the INCT-CA G 001/1 method described by Detmann et al. (2012). The total yields of TFDM, LBDM, SDM, and DMDM were assessed in kg ha^{-1}.

The efficiency of Mombaça grass was expressed as a function of the leaf blade dry mass. The partial efficiency values were obtained by calculating the ratio of LBDM (t ha^{-1}) to the amount of fertilizer applied in kg (N, P, and K present in the treatments) (Santos & Fonseca, 2016). Efficiency comprises the LBDM produced from the application of a given unit of fertilizer.

The values in *reais* necessary to produce one ton of LBDM were calculated as follows: [(total amount of reais spent on N + P + K in the treatment throughout the experimental period) / LBDM (t ha^{-1})] and expressed in reais (R$ / t of LBDM). In order to obtain the average values in reais of kg of fertilizers (for subsequent use in the calculation above), a market survey was carried out in the main input stores of the city of Parauapebas, Pará State. At least three stores were consulted to determine the average values in reais of the fertilizers urea (R$ 3.30), single superphosphate (R$ 2.40) and potassium chloride (R$ 3.20).

The data were subjected to descriptive analysis, and the assumptions of normal distribution and homoscedasticity for the variables were verified. The results were subjected to analysis of variance, considering the fertilization recommendations, days of N application, and the interaction between these two factors as sources of variation, according to the following model:

$$Y_{ijk} = \mu + b_k + E_i + D_j + (E.D)_{ij} + e_{ijk},$$

where Y_{ijk} is the value observed in the plot that received the factor E_i and D_j in block k; μ = general average; b_k= effect of the kth block; E_i = effect of the ith fertilization strategy on the response variable; D_j = effect of the jth day of N application on the response variable; $(E.D)_{ij}$ = effect of the interaction between fertilization recommendations × days of N application; and e_{ijk} = effect of the residual random error.

When significant effects were observed, the Tukey's test was used, and the interactions were split. Agroestat online software was used (Maldonado Junior, 2019). Differences were considered significant at $p \leq 0.05$. The number of cycles and the number of days per cycle were not statistically analyzed because they did not contain replications; only nominal values were presented.

Results

With the application of nitrogen fertilizer on the same day after defoliation of Mombaça grass (Day 0), the recommendations 5AP, RP, PG, and MM showed five, four, three, and five production cycles, respectively. When the application of nitrogen fertilizer occurred on the third (Day 3) and sixth days (Day 6) after defoliation, five, three, three, and three cycles were recorded, respectively, for the previously mentioned recommendations (Table 3).

Table 3. Total values of N, P2O5 and K_2O in kg ha^{-1}, applied throughout the experimental period as a function of the number of production cycles and average cycle duration of *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombaça grass.

	Fertilization Recommendations (kg ha^{-1})			
	5AP	RP	PG	MM
	Day 0			
kg ha^{-1} of N, P_2O_5 and K_2O	214.3- 70- 0	85.7- 16- 60	25.7- 80- 0	78.6- 10 -0
Number of Cycles	5	4	3	5
Interval between cycles (days)	27	30	43	31
	Day 3			
kg ha^{-1} of N, P_2O_5 and K_2O	214.3- 70- 0	64.3- 16- 60	25.7- 80- 0	47.1- 10- 0
Number of Cycles	5	3	3	3
Interval between cycles (days)	31	42	35	44
	Day 6			
kg ha^{-1} of N, P_2O_5 and K_2O	214.3- 70- 0	64.3- 16- 60	25.7- 80- 0	47.1- 10- 0
Number of Cycles	5	3	3	3
Interval between cycles (days)	27	41	42	42

5AP - recommendation based on the guide for the use of correctives and fertilizers of Minas Gerais - 5th Approach; RP - recommendation based on literature data applied to calculate nutrient replacement; PG - recommendation based on the fertilizer and liming guide of the state of Pará; MM - recommendation based on literature data (Mello et al., 2008) applied to the Michaelis-Menten equation.

Effects of the interaction between fertilization recommendations and timing of N fertilization on leaf appearance rate (LAR; $p < 0.0001$), leaf elongation rate (LER; $p = 0.002$), and leaf senescence rate (LSR; $p = 0.001$) are shown in Table 4.

Table 4. Mean morphogenic variables of *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombaça, under four fertilization recommendations in three periods (days) of N application.

Variables	Recommendations				Day of N application			SEM	P Value		
	5AP	RP	PG	MM	0	3	6		Rec.	Days	R x D
LAR	0.08	0.06	0.05	0.06	0.07	0.05	0.07	0.001	<0.0001	<0.0001	<0.0001
LER	4.79	3.38	2.71	3.50	3.97	3.03	3.78	0.10	<0.0001	0.0002	0.0020
SER	0.13a	0.09b	0.09b	0.08b	0.11A	0.10AB	0.08B	0.16	<0.0060	<0.003	0.0620
LSR	0.88	0.62	0.55	0.70	0.73	0.63	0.67	0.04	0.0020	0.1030	0.0010
LAI	5.33a	3.69b	3.23b	3.79b	4.06	4.13	3.84	0.02	0.0001	0.4340	0.1600
TPD	421.5a	249.1bc	212.0c	264.4b	292.5	279.4	288.4	0.16	0.0001	0.7780	0.1690

LAR = leaf appearance rate (cm day^{-1}); LER = leaf elongation rate (cm day^{-1}); SER = stem elongation rate (cm day^{-1}); LSR = Leaf senescence rate (cm/day); LAI = Leaf area index (m^2 m^{-2}); TPD = Tiller population density (tillers m^{-2}); 5AP = 5th Approach; RP = Nutrient Replacement; PG = Pará Guide; MM = Michaelis-Menten; SEM = Standard error of the mean; Different lowercase letters within rows indicate differences within fertilization recommendations; Different uppercase letters within rows indicate differences between the days of N application according to Tukey's test at the 5% probability level.

The highest LAR values were recorded for the recommendations 5AP (0.091 cm leaf $tiller^{-1}$ day^{-1}) and MM (0.078 cm leaf $tiller^{-1}$ day^{-1}) when N fertilization was performed on the same day after defoliation. In contrast, the 5AP recommendation (0.093 cm leaf $tiller^{-1}$ day^{-1}) with N applied on the sixth day after cutting had no significant effect ($p > 0.05$) (Figure 1A).

N fertilization on the third day after defoliation, and the 5AP recommendation caused the highest LAR (0.071 cm leaf $tiller^{-1}$ day^{-1}), which was 60.6, 70.25, and 61.8% higher than that for RP, PG, and MM, respectively. On the sixth day, values for RP (0.064 cm leaf $tiller^{-1}$ day^{-1}) and MM (0.062 cm leaf $tiller^{-1}$ day^{-1}) did not show differences (Figure 1A).

When the N fertilization was performed on the day of defoliation, the recommendations 5AP (5.24 cm day^{-1}) and MM (4.79 cm day^{-1}) showed similar LER values (Figure 1B). The lowest LER values were observed for the RP and PG, which were 3.36 cm day^{-1} and 2.52 cm day^{-1}, respectively (Table 4).

When N fertilization was performed on the third day after defoliation, the 5AP recommendation had values (4.12 cm day^{-1}) exceeding that for RP, PG, and MM by 68.7, 68.7, and 57.5%, respectively. When the fertilization was performed on the sixth day, LER values for recommendations 5AP (5.03 cm day^{-1}) and RP (3.96 cm day^{-1}) were similar to each other and higher than PG and MM values (Figure 1B).

There was no difference in LSR among N application treatments on the day of defoliation (Figure 1C). However, when N fertilization was performed on the third day after defoliation, LSR values for recommendations 5AP (0.95 cm $tiller^{-1}$ day^{-1}) and RP (0.68 cm $tiller^{-1}$ day^{-1}) did not differ from each other but were higher than MM (0.31 cm $tiller^{-1}$ day^{-1}). For N fertilization performed on the sixth day after defoliation, only RP (0.48 cm $tiller^{-1}$ day^{-1}) differed from MM (0.85 cm $tiller^{-1}$ day^{-1}) by 56.5%.

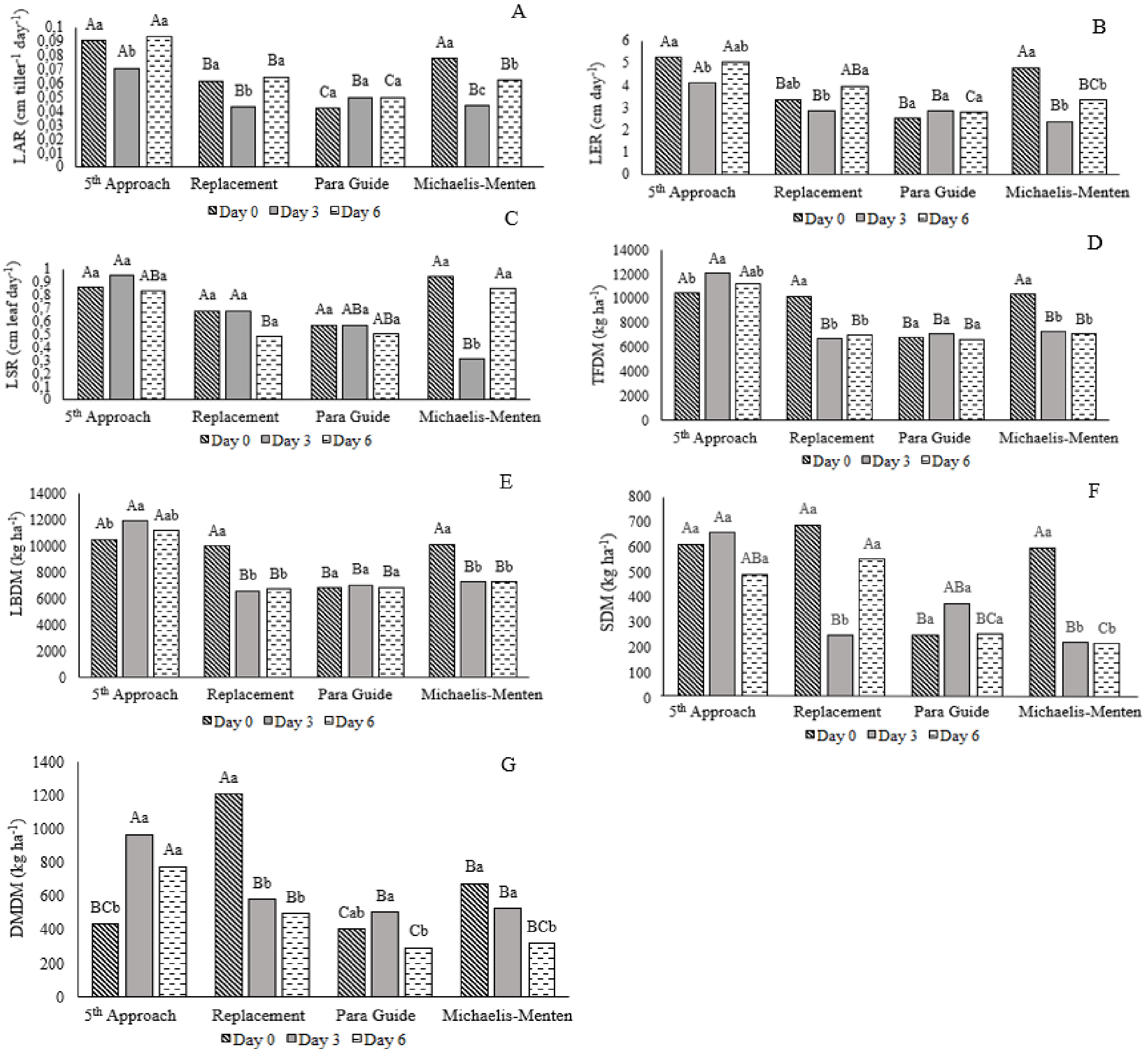

Figure 1. Mean LAR (A), LER (B), LSR (C), and yield of total forage dry mass - TFDM (D), total leaf blade dry mass - LBDM (E), total stem dry mass - SDM (F) and total dead material dry mass (G) in kg ha^{-1} of Megathyrsus maximus (syn. Panicum maximum) cv. Mombaça grass under different fertilization recommendations in three periods (days) of N application after defoliation. Different uppercase letters indicate difference between fertilization recommendations on a given day of N application and different lowercase letters indicate difference between application days on a given fertilization recommendation.

Both fertilization recommendations (p = 0.006) and the timing of N fertilization affected SER (p = 0.003) (Table 3). When analyzing the effect of the recommendations, the highest SER was observed for 5AP (0.133 cm tiller-1 day-1), which was 32.5, 34.7, and 41.1% higher SER than RP, PG, and MM, respectively, and the latter three were similar in values. In addition, when nitrogen fertilization was performed on the day of defoliation, it promoted greater SER (0.113 cm tiller-1 day-1) compared to the fertilization performed on the sixth day (0.077 cm tiller-1 day-1) (Table 4).

Leaf area index (LAI) and tiller population density m-2 (TPD) were affected only by fertilization recommendations (p = 0.0001) (Table 3). The 5AP recommendation showed an average of 5.33 m^2 of leaf blades per m-2 of soil, which was superior by 28.9, 30.8, and 39.4% compared to MM (3.79), PR (3.69), and PG (3.23), respectively.

The highest tiller emission per m2 (421.5) was observed for the 5AP recommendation, which was superior to those of PG, PR, and MM by 49.7, 40.9, and 37.3%, respectively. The value for MM (264) was superior to that for PG (212).

There was an interactive effect between fertilization recommendations and the timing of nitrogen fertilization on TFDM (p = 0.0001), LBDM (p = 0.0001), DMDM (p = 0.0001), and SDM (p = 0.0008) (Table 5).

The highest TFDM (12,109 kg DM ha^{-1}) was recorded in case of the 5AP recommendation with nitrogen fertilization on the third day after defoliation (Figure 1D). When nitrogen fertilization was performed immediately after defoliation, the PG recommendation showed the lowest TFDM (7,473 kg DM ha^{-1}) among all recommendations. The other recommendations did not differ significantly ($p > 0.05$) from each other (11,538, 11,193, and 11,405 kg DM ha^{-1} for 5AP, RP, and MM) (Figure 1D).

Table 5. Mean yield variables of *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombaça under four fertilization recommendations at three moments (days) of N application.

Variables	Recommendations				Day of N application			SEM	P Value		
	5AP	RP	PG	MM	0	3	6		Rec.	Days	R x D
TFDM	12,607.95	8,793.86	7,598.96	9,084.66	10,402.23	9,304.59	8,857.26	5.46	0.0001	0.0001	0.0001
LBDM	11,197.00	7,758.70	6,902.20	8,231.00	9,350.90	8,208.90	8,007.00	0.01	0.0001	0.0001	0.0001
DMDM	726.44	762.72	402.14	508.23	681.97	645.81	471.87	0.37	0.0001	0.0002	0.0001
SDM	587.3	496.89	294.59	345.41	537.71	378.38	377.06	2.22	0.0001	0.0042	0.0008

TFDM = total forage dry mass yield ha^{-1}; LBDM = total leaf blade dry mass yield ha^{-1}; DMDM = total dead material dry mass yield ha^{-1}; SDM = total stem dry mass yield ha^{-1}; 5AP = 5th approach; RP = nutrient replacement; PG = Pará Guide; MM = Michaelis-Menten; SEM = standard error of the mean.

Similar to the TFDM, the LBDM showed higher yield (11,919 kg ha^{-1}) when nitrogen fertilization was performed on the third day after defoliation, in comparison to the fertilization immediately after defoliation in case of the 5AP fertilization recommendation (Figure 1E). PG showed the lowest LBDM (6,815.5 kg DM ha^{-1}) among all treatments when nitrogen fertilizer was applied immediately after defoliation. The other recommendations did not differ among themselves ($p > 0.05$).

The SDM value showed no change when nitrogen fertilization was applied immediately after defoliation, except for PG, which showed the lowest value. When the fertilization occurred on the third day, the 5AP recommendation produced the highest amount of stem mass (658.06 kg ha^{-1}), which was higher than RP (251.38 kg ha^{-1}) and MM (222.56 kg ha^{-1}). In addition, the recommended PG showed the least variation between the days of N application, with no effect on SDM as a function of fertilization timing. When the fertilization was performed on the sixth day after defoliation, the highest SDM was observed for the RP recommendation (550.7 kg ha^{-1}), which was higher than that for PG (255.1 kg ha^{-1}) and MM (215.7 kg ha^{-1}) by 46.3 and 39.2%, respectively (Figure 1F).

When the fertilization was performed right after defoliation, the RP recommendation produced the highest amount of DMDM (1,209.6 kg ha^{-1}), followed by MM (676.63 kg ha^{-1}), which was higher than PG (405.4 kg ha^{-1}) (Figure 1G). When performing fertilization on the third day after defoliation, the 5AP recommendation showed the greatest DMDM value (967.31 kg ha^{-1}), while the others PR, PG, and MM showed no significant differences. The application of 5AP recommendation with fertilization on the sixth day showed the greatest DMDM value (775.72 kg ha^{-1}), followed by that for PR (498.17 kg ha^{-1}). The PG treatment (293.6 kg ha^{-1}) had the lowest average of DMDM (Figure 1G).

In terms of the fertilizer-use efficiency of Mombaça grass (Table 6), the highest LBDM value was observed for the 5AP recommendation with fertilization on the third day after defoliation (5AP3), followed by MM with fertilization on day 0 (MM0), RP with fertilization on day 0 (RP0), and PG, which caused no differences in LBDM between the days of N application, presenting very close values for R$/ t of LBDM ha^{-1} (R$ 188.17 193.36).

Table 6. Use efficiency of N, P and K fertilizers supplied during the whole experimental period to the treatments, given in R$/ton of LBDM.

Treatments	Fertilizers cost (R$ t^{-1} of LBDM ha^{-1})					
	Urea 45%	S.S 17%	KCl 60%	NPK Cost (R$)	LBDM Yield (t ha^{-1})	Efficiency (R$/ t LBDM)
5AP0	476.22	411.76	0	R$2,559.77	10.48	R$244.25
5AP3	476.22	411.76	0	R$2,559.77	11.452	R$223.52
5AP6	476.22	411.76	0	R$2,559.77	11.15	R$229.58
RP0	190.44	94.12	100	R$1,174.35	9.784	R$120.03
RP3	142.89	94.12	100	R$1,017.42	6.5899	R$154.39
RP6	142.89	94.12	100	R$1,017.42	6.6422	R$153.17
PG0	57.11	470.59	0	R$1,317.88	6.8155	R$193.36
PG3	57.11	470.59	0	R$1,317.88	7.0036	R$188.17
PG6	57.11	470.59	0	R$1,317.88	6.8571	R$192.19
MM0	174.67	58.82	0	R$717.58	10.023	R$71.59
MM3	104.67	58.82	0	R$486.58	7.292	R$66.73
MM6	104.67	58.82	0	R$486.58	7.2705	R$66.92

(S.S) Single superphosphate, 17% of P_2O_5; (KCl) potassium chloride, 60% of K_20; (LBDM) leaf blade dry mass; 5AP = 5th approach; RP = nutrient replacement; PG = Pará guide; MM = Michaelis-Menten; *The numbers followed by the abbreviations represent the day when nitrogen fertilization was applied after defoliation.

Discussion

The increase in N in the soil-plant system provided by the 5AP recommendation favored a high LAR after defoliation (Martuscello et al., 2005; Patês et al., 2007). The similarity of the results of RP and MM recommendations occurred due to a smaller difference in the N levels under both treatments, i.e., 85.7 and 78.6 kg, respectively. The MM recommendation showed LAR similar to that of 5AP on day 0.

LAR marks a centrally important response in morphogenesis, as it directly influences each of the structural components, and consequently, the leaf area index of the pasture (Lemaire & Chapman, 1996; Alexandrino, Nascimento Júnior, Mosquim, Regazzi, & Rocha, 2004; Difante et al., 2011). According to Martuscello et al. (2015), a higher LAR may be indicative of an increased number of leaves tiller^{-1} and consequent gains in biomass yield.

N-deficient soils usually limit plant growth, with N and P required for the biosynthesis of abundant and essential biomolecules, such as proteins and nucleic acids, respectively (Rajasekar, Nandhini, Swaminathan, & Balakrishnan, 2017). The PG recommendation had the lowest N concentration per application, thus causing the lowest LAR, which corroborates the previous statement that N increment increases LAR in forage plants. The P content of PG, which was 80 kg ha^{-1} of P_2O_5, was the highest among the treatments. However, this was not a decisive factor in the increase in LAR, and probably, the low N availability limited the use of P by the plant.

Evaluating the application of a fixed level of 90 kg N ha^{-1} and 60 kg K_2O ha^{-1} and varying only the levels of P_2O_5 (0, 30, 60, 90, and 120 kg ha^{-1}) in *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombaça, Costa et al. (2017) reported a quadratic response for LAR, with a maximum point of 0.109 leaf day^{-1} with the application of 60 kg P_2O_5. These values were close to those obtained in the present study for the 5AP recommendation when N was applied on days 0 and 6. We also observed the relevance of the association of N with P for a better response of forage plants.

Regarding the leaf elongation rate (LER), higher levels of N supplied by the 5AP recommendation caused the plant to present the most favorable moment for fertilizer application on the day of defoliation. It is possible that on the third day after cutting the plant, when N fertilizer was applied, it could respond physiologically to the stress of defoliation. For this reason, N fertilization on the third day for the 5AP recommendation led to reduced LER compared with that on day 0.

In the present study, the greatest stress caused to the plant did not seem to have been on the day of defoliation because the MM recommendation with N fertilization on the day of defoliation showed LER similar to that presented by the treatments of the 5AP recommendation. In fact, LER is an important measure in the analysis of plant tissue flow and has a positive relationship with forage yield, promoting greater accumulation of dry matter due to the increased proportion of leaves and photosynthetically active areas (Martuscello et al., 2006; Paciullo et al., 2017).

According to Marques et al. (2016), N fertilization has a direct and marked effect on the photosynthesizing leaf area as a consequence of the increase in the LER. However, it is worth mentioning that the 5AP recommendation provided at the beginning of the evaluation was 70 kg ha^{-1} of P_2O_5, and the MM recommendation provided only 10 kg of P_2O_5 ha^{-1}, which shows that the participation of P in the morphogenic responses was less evident in this study.

The lower LER observed for 5AP, RP, and MM recommendations on the third day after defoliation may have occurred because of the cut, which would have stimulated the root metabolism of the plant to translocate photoassimilates to the shoot to recover the leaf area that is responsible for photosynthesis. At this moment, the plant would have a lower capacity to absorb nutrients, such as N. However, this behavior was not observed for the PG recommendation, possibly due to its low N level.

A higher SER may be a response to the higher level of N provided by the 5AP recommendation. Braz et al. (2011) and Iwamoto et al. (2015) also observed this behavior when evaluating *Megathyrsus maximus* cv. Tanzania was subjected to higher levels of N. Higher SER can also be caused by the shading of the upper leaves over the leaves at the plant's base, according to Silva and Nascimento Junior (2007). Although each forage species has its own genetic programming, SER can be intensified with increased competition for light. Therefore, the results of LAI and TPD obtained in this study may explain the higher SER in the 5AP recommendation, as these variables were higher in this recommendation.

Pasture N fertilization has a positive effect on leaf blade average length (LBAL) (Pereira et al., 2011), because this nutrient significantly increases LER compared to the stimulus by LAR (Martuscello et al., 2006; Premazzi, Monteiro, & de Oliveira, 2011). However, according to Volaire, Barkaoui, and Norton (2014), final leaf blade length is a plastic trait that responds to defoliation intensity. Therefore, it is possible that no differences in LBAL were found, since all treatments had the same residue height (30 cm).

Studies on tropical forages, especially those with *Megathyrsus maximus* (syn. *Panicum maximum*) reported the influence of higher N availability on increased LSR (Iwamoto et al., 2015; Martuscello et al., 2015; Santos & Fonseca, 2016; Martuscello et al., 2019). However, this response was not observed in the present study.

The emission of tillers was stimulated by the use of increased levels of N in the 5AP recommendation, which suggests that the timing of N fertilization up to the sixth day after defoliation does not influence tillering like the amounts of N and P available to the pasture do. The increase in tillering is closely related to N fertilization, as it is linked to an increase in cell recycling, with the appearance and growth of tissues, including basilar and axillary buds, which are precursors of tillers (Martuscello et al., 2015). Each time a new leaf is formed, an axillary bud also forms, which can develop into a new tiller (Santos & Fonseca, 2016). The 5AP recommendation, with a total of 214.3 kg of N ha^{-1}, produced the most leaf blades, and consequently a greater number of axillary buds, which explains the higher tiller emission.

LAI showed a similar behavior, probably due to greater tillering. The isolated effect of the recommendations resulted in a greater LAI in 5AP, possibly due to the greater level of N applied to the plants. The greater leaf area shows evidence of the promoting effect of cell division influenced by N, which stimulates an increase in the production of new cells, in the rates of leaf appearance and elongation (Martuscello et al., 2005). Notably, the level of 70 kg of P_2O_5 ha^{-1} (30.57 kg P ha^{-1}) preconized by the 5AP recommendation, associated with the N supplied (214.3 kg ha^{-1}), may have promoted better utilization by the plants. Such factors in addition to higher tillering may translate into an increase in the LAI.

A greater TPD associated with a greater LAI provides faster plant recovery after defoliation. It is possible that the residue of defoliation has a greater residual leaf blade area, which results in better conditions for regrowth in shorter periods.

From the information obtained in this study, it was observed that the level of N was the most influential for the variations in LAR and LER for the 5AP, RP, and MM recommendations. This caused the plants to present the greatest responses for these recommendations, with similar behavior when N fertilization was performed on days 0 and 6. In Figures 1A and 1B, it is observed that the fertilization performed on the third day after defoliation promoted a reduction in the values of LAR and LER. This because on day 0, the plant had not yet physiologically responded to the stress of defoliation.

The reduction in LAR on day 3 may be related to the plant's response to defoliation, and on day 6, there may have been plant recovery from harvest stress, as on that day the plant presented the largest leaf area. Santos, Thornton, and Corsi (2012) stated that defoliation reduces root activity in *Panicum maximum*, and according to the authors, the species is mainly dependent on N uptake by the root to ensure growth after defoliation.

The PG recommendation was the only one that did not cause differences in LAR and LER between fertilization stages. Despite the 80 kg of P_2O_5 made available, owing to the low level of N recommended by the guide, its effect on the forage may have been innocuous, being indifferent to the day of fertilization.

Evaluation of *Megathyrsus maximus* cv. Faria et al. (2019) tested different moments of N fertilization after defoliation and found greater accumulation of root DM when fertilization occurred on the sixth day after defoliation. Although the study was carried out in a greenhouse, this information may explain, in part, the better performance of LAR and LER in the present study for the 5AP, RP, and MM recommendations on the sixth day in comparison to the fertilization performed on the third day after defoliation.

Another factor that should be noted was the number of cycles during the evaluation period. Only the 5AP recommendation (at the three N fertilization moments) and MM recommendation (fertilization on day 0) provided five cycles. Proper pasture management can be obtained by the number of more frequent defoliation cycles (Zanine et al., 2013), as SER and LSR are affected by the frequency of defoliation, in which a higher frequency implies lower rates (Zanine et al., 2018), which depreciates the nutritive value and decreases animal performance. Therefore, the MM recommendation with fertilization on the day of defoliation showed a lower stem elongation rate, which may result in forage of better quality.

Similar values were observed in treatments 5AP3 and 5AP6 for TFDM, possibly due to the higher amount of N applied after defoliation. However, one should evaluate the importance of P, which together with N are necessary for the biosynthesis of abundant and essential biomolecules, such as proteins and nucleic acids, respectively (Rajasekar et al., 2017).

By acting as a source of photoassimilates for new leaf emissions, the plant probably limited the utilization of the maximum N supplied. Defoliation affects the specific N uptake by the root, and therefore, the potential for uptake to supply growing leaves (Thornton & Millard, 1997). However, in the present study, it was not possible to determine the exact time that this occurred, but it is likely that it occurred between the day of defoliation and the third day, which explains the higher TFDM in the 5AP recommendation with N fertilization on days three and six after defoliation, when the plant had sufficient leaf area to alter the source-drain relationship and allow greater N uptake by the root.

When the RP and MM recommendations were analyzed, the best responses were obtained when the N source was applied on day 0. It is possible that N helped the plant re-establish itself soon after defoliation.

The PG recommendation was the only one in which the timing of N fertilization did not influence the dry matter yield (TFDM). The low amount of N supplied was the limiting factor despite supplying a good amount of phosphorus (P). This mineral, in turn, in the amounts provided by the recommendations, did not limit plant growth.

The average proportion of leaves in the morphological composition for all treatments was 88.7%. The 5AP recommendation, regardless of the timing of N fertilization, presented the highest LBDM. Therefore, this result shows that when this recommendation is adopted, it is possible to work with greater flexibility in the application of N fertilizer, but the efficiency of use of the inputs by the plant must be taken into consideration.

Fertilization with N is related to greater protein production because it provides more N for protein synthesis and enhances the development of leaf blades (Alexandrino et al., 2004). Moreover, the use of higher levels of N, as in the case of the 5AP recommendation, stimulates greater tiller emission than the other recommendations (RP, PG, and MM). This also explains the higher LBDM yield in treatments with the 5AP recommendations.

From the results presented in this study, it is evident that using the RP and MM recommendations, the timing of fertilization has a more intense influence on the production of leaf blades. The greatest elasticities in production were observed when the N level was lower, which was noticeable for the RP and MM recommendations with fertilization on day 0, with total levels of 21.42 and 15.72 kg of N ha^{-1} $cycle^{-1}$, and there was greater productivity for fertilizations on the third or sixth day after defoliation.

It is possible that physiological stress after defoliation was triggered after day 0, unlike in plants treated with 5AP. Therefore, it can be suggested that for the recommendations that used lower levels of N (RP and MM), the plants did not immediately decrease the uptake of nutrients from the soil, therefore, even in smaller amounts, the N applied on day 0 stimulated the plants to expand their leaf area and recover more quickly from a possible deficit of assimilates.

In general, SDM was reduced in this study. The average of the treatments was 4.5% of the total harvested dry mass, which can be seen as a positive factor because the stem has a lower nutritional value than the leaf blade. Stem elongation occurs when the LAI is above the critical level; therefore, it is a plant strategy to intercept light of high quality and quantity in the lower leaves (Alexandrino, Gomide, & Gomide, 2005). It is likely that in this study, the plants did not reach the critical LAI because of the lower proportion of stems in the material harvested up to 30 cm from the ground level.

SDM yield may be correlated with higher N input to the soil, which stimulates a higher number of production cycles. Therefore, in the treatments with five production cycles, the three fertilization recommendations with 5AP and MM on day 0 and RP on day 0 with four cycles showed higher stem yields. The same pattern was observed for dry matter and leaf blade yield.

Regarding the amount of DMDM, the literature explains that higher levels of fertilizers, especially nitrogen, increase tillering, parallel leaf size, and LAI (Martuscello et al., 2005). This response to nitrogen fertilization causes the plant to present greater shading of the forage canopy, and the older leaves and those at the base of the stem tend to decrease the photosynthetic rate and begin senescence, increasing the amount of dead material (Santos et al., 2011).

The treatments that adopted the 5AP recommendation obtained the highest DMDM values, which occurred with N fertilization on the third and sixth days after defoliation. However, with fertilization immediately after cutting (day 0), the 5AP recommendation, which was expected to yield similar results to those obtained on the third and sixth days due to good forage mass yield and higher LAI, showed one of the lowest values of DMDM followed by PG and MM. In fact, it is likely that although the LAI value was higher than the other fertilization recommendations, it did not reach a value close to the critical LAI due to the low DMDM.

Based on the agronomic variables, it was observed that the 5AP recommendations at the three periods of N application, and MM and RP with N application on the day of defoliation, showed the highest TFDM. However, MM, from the point of view of fertilizer use efficiency and saving of financial resources, exceeded 5AP, as the cost of fertilizers to produce one ton of DM was 3.56 times lower (Table 5).

Conclusion

To obtain a greater number of production cycles and higher absolute values of leaf blade dry mass (LBDM), the 5AP recommendation should be adopted with N application on the same day and/or on the sixth day after defoliation and the MM recommendation with N application on the same day of defoliation. In contrast, the best LBDM production efficiency of LBDM is obtained with the MM recommendation with N fertilization on the same day of defoliation. It is also worth mentioning that different recommendations present diverse results and should be applied according to the reality of each production system.

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