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Nitrogen fertilisation as a strategy for intensifying production and improving the quality of Massai grass grown in a humid tropical climate

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ABSTRACT

The use of nitrogen fertilizers intensifies forage production, especially in tropical climates. This study aimed to evaluate the effects of nitrogen fertilization on the quantitative and qualitative characteristics of Massai grass grown in a humid tropical climate, specifically Am-type. A completely randomized design was used with six treatments and five replicates. The treatments used were a control treatment (without nitrogen application) and five doses of nitrogen fertilization (100, 200, 300, 400, and 500 kg N ha^{-1} $year^{-1}$). Urea was used as the nitrogen fertilizer for six applications, with an interval of 30 days. The leaf elongation rate increased by 69.1% at a dose of 500 kg N ha^{-1} compared to that of the control. The leaf appearance rate and green leaves per tiller increased with nitrogen fertilization. Nitrogen fertilization did not affect the percentage of leaf blades and dead tissue. The average height of the canopies was 44.0 cm, regardless of fertilization. There was a quadratic response in total forage accumulation (TFA), with a maximum of 19,136 kg dry matter at a dose of 391 kg N $ha^{-1}year^{-1}$. Nitrogen utilization efficiency was the same as that of TFA. There was a reduction in the recovery of applied nitrogen and the agronomic efficiency of applied nitrogen with nitrogen fertilization. The number of cuts increased linearly with nitrogen fertilization, from 3.2 to 7.5 cuts. Crude protein content increased with nitrogen fertilization. Therefore, nitrogen fertilization favored the productivity and quality of Massai grass grown in a humid tropical climate.

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fodder; forage accumulation; leaf appearance rate; *Megathyrsus maximus* × *M. infestus*; nitrogen; sheet

Introduction

The deforestation process continues to advance over important areas of rainforest biomes worldwide. In Brazil, deforestation in the Legal Amazon increased from 6,207 km^2 in deforested areas in 2015 to 11,088 km^2 in 2020 (INPE 2021), showing an increase of 78.64% over the last five years. Historically, the expansion of livestock activity has been one of the main causes of deforestation. The best example in the Brazilian Amazon is extensive cattle ranching, which is highly concentrated in land use and accounts for more than 60% of deforestation in the region (Almeida et al. 2016).

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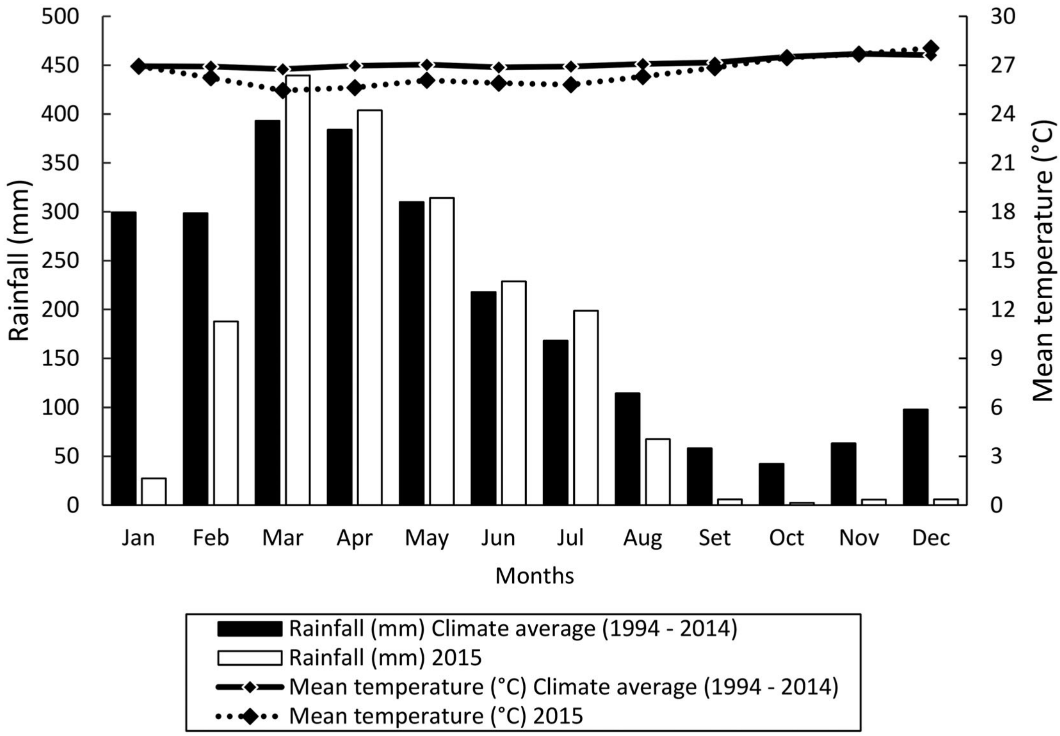

Figure 1. Average monthly rainfall (mm) from 1994 to 2014 and 2015, and average temperature (° C) from 1994 to 2014 and 2015 in the municipality of Igarapé-Açu, Pará.
Source: Data for the average between 2004 and 2014 from the EMBRAPA meteorological station and the data for 2015 from the Igarapé-Açu School Farm meteorological station.

The use of technologies that increase land use efficiency is fundamental for decreasing the deforestation rate to the detriment of the opening of new areas for livestock exploitation (Silva et al. 2018). Studies have shown that the intensification of livestock in areas bordering the biomes already mentioned indicates the possibility of decreasing global greenhouse gas emissions and protecting deforestation areas (Cohn et al. 2014; Strassburg et al. 2014). Nitrogen fertilization is an alternative method for increasing productivity in livestock systems (Macdonald et al. 2017; Delevatti et al. 2019). Nitrogen stimulates plant growth in most ecosystems (Lebauer and Treseder 2008), which in turn can fix carbon in the biomass.

The effect of nitrogen fertilization is due to the increased production of cells that, under favorable physiological conditions, accelerates growth, tillering, and leaf production (Dupas et al. 2010; Batista et al. 2014). In addition, it promotes cell division, which promotes an increase in the rate of appearance and elongation of leaves, which are responsible for forage yield (Volenec and Nelson 1983; Dupas et al. 2010; Batista et al. 2014). Thus, if used at the correct time of the year, nitrogen fertilization can anticipate the moment of defoliation of the plant, shortening the rest periods to which the pasture is subjected, especially the tropical climate.

Thus, the effect of nitrogen on the growth of tropical grasses, such as Massai (*Megathyrsus maximus* × *M. infestus*), can increase the regrowth capacity because, after defoliation, rapid recovery of the photosynthetic apparatus can enable the survival of the plant canopy. In addition, nitrogen plays an important role in the nutritional quality of forage plants by increasing the levels of crude protein and total digestible nutrients levels (Kering et al. 2011). However, the effect of nitrogen fertilization on crops will only be enhanced if it is done at a favorable time of year for precipitation, light, and temperature.

Field studies to measure and understand the growth dynamics of plants under intensive management conditions, and exploration of the manifestation of a range of variables, including morphogenic expression, nutrient use efficiency, and chemical composition of plants, are essential. We hypothesize that these variables are dose dependently affected by application of nitrogen fertilizer and that the relationship between these variables and nitrogen dosage can inform the adoption of strategies for more efficient and sustainable fertilizer use. Thus, the objective of the present study was to determine the effect of nitrogen fertilization on structural and morphogenic characteristics, growth dynamics, production, and efficiency during the fertilization process, and the chemical composition of Massai grass grown in a humid tropical climate, specifically an Am-type climate.

Materials and methods

Experimental site and sowing of seed

The experiment was performed at the Igarapé-Açu School Farm in Igarapé-Açu, Pará, belonging to the Federal Rural University of the Amazon (01°07′21″ S, 47°36′27″ W; altitude 47 m above sea level). Before the beginning of the experiment, the vegetation cover was grasses, secondary vegetation, or scrubs. The area was considerably altered in terms of the original vegetation, and there had never been any fertilization had never previously taken place. The topography of the area is flat and homogeneous. The Igarapé-Açu region has an average annual temperature of 26.8 °C (Figure 1) and an Am-type climate, according to the Köppen classification (Alvares et al. 2013), characterized by a rainy climate, with a short dry season.

The soil in the experimental area was classified as a yellow latosol with a sandy loam texture and low fertility. Chemical analysis of the soil was performed in January 2014 with the following results: pH in H_2O: 4.7; M.O: 7.98 g kg^{-1}; P: 1.5 mg dm^{-3} (Mehlich-1) and K: 0.03 cmolc dm^{-3}; Ca^{2+}: 0.28 cmolc dm^{-3}; Mg^{2+}: 0.28 cmolc dm^{-3}; and Al^{3+}: 1.11 cmolc dm^{-3} (KCl 1 mol L^{-1}). In the formation of the experimental area with Massai grass, the soil was corrected with dolomitic limestone (TNRP 95%). A dose equivalent to 2.9 ton ha^{-1} was applied to increase the base saturation to 60%.

After soil preparation on 20 May 2014, sowing was performed with Massai grass seeds, together with phosphate fertilization (simple superphosphate, with 18% P_2O_5), a dose equivalent to 80 kg of P_2O_5 ha^{-1}, applied by haul. The plant community of the plots remained in free growth until they reached approximately 50 cm in height. They were then cut periodically with high intensity, at a residual height of 15 cm above the ground, whenever the forage canopy reached 95% of light interception.

In January 2015, new phosphate fertilization was performed with the same amount of P_2O_5. During potassium fertilization, a dose equivalent to 60 kg K_2O ha^{-1} was added together with nitrogen fertilization. The experiment was performed during the highest rainfall period and began on 14 February 2015 and ended on 31 August 2015.

Treatments and experimental design

A completely randomized design was used, with a control treatment (without nitrogen application) and five doses of nitrogen fertilization in Massai grass, with five replications. The doses corresponded to 100, 200, 300, 400, and 500 kg N ha^{-1} $year^{-1}$, using agricultural urea as a nitrogen source during the rainy season. The plots were 12 m^2 (3 m × 4 m) and were separated by 1 m wide corridors. There was a border area of 1 m from the ends of the plots where the material and data were not collected.

Nitrogen and potassium fertilization were divided into six applications with an interval of 30 days during the months that presented precipitation higher than 150 mm, which was determined by observing the average annual precipitation from 1994 to 2014 (Figure 1). Applications were conducted on the following dates: 14 February, 16 March, 15 April, 15 May, 14 June, and 14 July 2015. The first application was undertaken after leveling all the plots on the first day of the experiment, and the others were performed on fixed days.

Experimental measurements

After leveling, five tillers were marked in each plot, choosing the tiller where the vegetation condition was representative of the average condition of the plot. The tillers were identified with plastic clamps, listed, and measured using a graduated ruler. Because of the accelerated renewal of tissues due to the high rainfall, temperature, and light quality of the region, the tillers were monitored every two days. New tillers were marked at each cut of the plot, following the same initial criteria.

To evaluate the morphogenic and structural characteristics, the leaves were numbered and classified as completely expanded leaves (fully exposed and visible leaflet), expanding leaves (without a visible leaflet), and senescent leaves (when the leaf blade tip showed any indication of senescence-yellowing). Leaves in which more than 50% of the leaf blade length were senescent were considered dead. The length of the stalk was measured from the ground level to the last fully expanded leaflets (Duru and Ducrocq 2000). To aid measurements, a graduated ruler was used and the data were recorded in previously prepared spreadsheets containing spaces for date, stem and leaf lengths, and a legend with the letters E, Ex, and S indicating the state of the leaf, that is, expansion, expansion, or senescence, respectively.

After these evaluations, it was possible to determine the following morphogenic and structural variables: (i) leaf appearance rate (LAR), expressed in cm tiller^{-1} day^{-1} leaves, which was determined by the average number of leaves per tiller divided by the number of days in the evaluation period; (ii) leaf elongation rate (LER), expressed in cm tiller^{-1} day^{-1}, which was determined by the average variation in the length of leaf blades in expansion per tiller divided by the number of days in the evaluation period; (iii) phyllochron (PHILO), expressed in leaf^{-1} tiller^{-1} days, which is the inverse of the LAR, i.e. PHILO $=$ 1 LAR^{-1}; (iv) leaf senescence rate (LSR), expressed in cm tiller^{-1} day^{-1}, which was determined by the average variation in the length of the senescent portion of the leaf blade per tiller divided by the number of days in the evaluation period; (v) stem elongation rate (SER) in cm tiller^{-1} day^{-1}, which was the average variation in stem length (stalk $+$ pseudholm) determined by tiller divided by the number of days in the evaluation period; (vi) initial stem length (ISL) and final stem length (FSL), expressed in cm, were determined by the distance between the ligula of the last expanded leaf related to the soil or the insertion in the original tiller, in the case of aerial tillers; (vii) green leaves per tiller (GLT), obtained from the average number of leaves in expansion, expanded, and in senescence per tiller, excluding the leaves that presented more than 50% of the leaf blade in the senescence process; (viii) number of senescent leaves (NSL), obtained from the average number of senescent leaves; (ix) number of fully expanded leaves (NFEL), average leaf count that presented the ligula, being completely expanded; and (x) leaf lifespan (LLS), expressed in days, determined by multiplying the number of live leaves by the PHILO.

The canopy was always cut when the plot reached 95% light interception (LI). This condition was determined by sampling five points in the plot to obtain an average LI value using a linear quantum sensor (Ceptometer LP-80; Decagon Devices, Inc., Pullman, WA, USA). In addition, the average canopy height was measured at five points per plot, using a graduated stick with a polyacetate sheet. Forage samples were collected per plot, at the end of each regrowth cycle, to determine the forage accumulation per cut (FAC) in kg of dry matter (DM) ha^{-1}, daily forage accumulation (DFA) in kg of DM ha^{-1} day^{-1}, and the total forage accumulation (TFA) in kg of

DM ha^{-1} ano^{-1}. The forage contained within a metal frame was collected, with dimensions of 1.0 m × 0.5 m and a height of 15 cm above ground level (residual height). Soon after collection, the samples were immediately packed in plastic bags, identified, weighed, and cooled (−20 °C) for future processing.

After thawing, the samples were weighed and homogenized. One sub-sample was removed and used to separate the morphological composition, and the other sub-sample was weighed and dried to determine the mass contained in the frame and the chemical composition of the forage. The morphological components were separated into three portions: leaf blade (LB), stem and sheath (S) (the inflorescences when present were kept in that portion), and dead tissue (DT). The portions and samples of the entire plant were weighed again, placed in paper bags, and dried in a forced circulation oven at 55 °C for 72 h. Subsequently, the entire plant samples were weighed to determine the forage mass (FM).

The proportion of each morphological component was expressed as a percentage of the total weight of the sample. The sum of the leaf blade and stem (LBS), expressed in kg ha^{-1}, was determined. The weights of the morphological components were used to determine the LB: stem ratio (LSR), determined by dividing LB by S.

The FAC was determined as the FM harvested above 15 cm, measured in 0.5 m^2 of the plot, extrapolated to FM in one hectare. To quantify the DFA, the ratio between the DFA and the number of rest days (the period between a cut and the time that the canopy needed to reach 95% LI) was calculated. TFA was determined by adding the FM ha ha^{-1} to all cuts obtained during the experimental period. Based on this information, other evaluation parameters were determined, such as leaf blade and stem accumulation (LBSA), leaf blade accumulation (LBA), and stem accumulation (SA), calculated from the TFA value regarding the percentage of these components in the sample.

The sub-samples used for FM quantification after drying at 55 °C were ground in a knife mill, and transferred through a sieve with 1 mm porosity to determine the chemical composition. For the determination of DM, the samples were dried in an oven at 105 °C for 16 h (AOAC 1990; method 967.03). The ash content was determined by incinerating organic matter (OM) at 600 °C for 4 h in a muffle furnace (AOAC 1990; method 942.05).

Crude protein (CP) analyses were performed according to the AOAC (1990) (method 984.13). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) levels were analyzed sequentially using ANKOM-type equipment with procedures outlined by ANKOM Technology Corp. (Van Soest, Robertson, and Lewis 1991; Vogel et al. 1999), without the use of sodium sulfite and thermostable α-amylase in the neutral detergent solution. Hemicellulose (HEM) content was determined based on the difference between NDF and ADF.

Nitrogen content was determined according to the AOAC (1990) (method 984.13) .The nitrogen accumulation (NA) values were obtained by multiplied nitrogen content by total forage accumulation. The following indices were calculated using dry mass and NA data:

- Nitrogen utilization efficiency (NUE): Takes in account absolute biomass increase (Siddiqi and Glass 1981; Good, Shrawat, and Muench 2004).
- NUE (kg^2 g^{-1}) = (TFA, kg)^2/(NA, g);
- Recovery of applied nitrogen (RAN): Measures the efficiency of nitrogen capture from soil (Craswell and Godwin 1984; Good, Shrawat, and Muench 2004).
- RAN (%) = (NA with fertilization, kg – NA without fertilization, kg) × 100/(fertilizer N applied, kg);
- Agronomic efficiency (AE) measures the efficiency of converting applied nitrogen to biomass yield (Craswell and Godwin 1984; Good, Shrawat, and Muench 2004).
- AE (kg kg^{-1}) = (TFA with fertilization, kg – TFA without fertilization, kg)/(fertilizer N applied, kg);

Table 1. Morphogenic and structural characteristics of Massai grass.

Characteristics evaluated	Doses of N (kg ha^{-1}) 0	100	200	300	400	500	*p-value*	Regression Equation	r^2
LER (cm tiller^{-1} day^{-1})	1.909	2.568	3.060	3.547	3.592	3.685	<0.0001	$Y = 2.17 + 0.003x$	0.73
LAR (number of leaves tiller^{-1}day^{-1})	0.068	0.083	0.091	0.109	0.117	0.124	<0.0001	$Y = 0.07 + 0.0001x$	0.85
PHILO (days leaf^{-1}tiller^{-1})	13.7	13.6	11.5	9.9	9.2	8.7	<0.0001	$Y = 13.97 - 0.01x$	0.76
LLS (days)	52.6	48.3	43.9	39.1	36.1	35.0	<0.0001	$Y = 51.72 - 0.03x$	0.96
LSR (cm tiller^{-1} day^{-1})	0.099	0.112	0.116	0.150	0.205	0.270	<0.0001	$Y = 0.07 + 0.0003x$	0.57
SER (cm tiller^{-1} day^{-1})	0.030	0.033	0.057	0.072	0.058	0.051	0.0093	$Y = 0.025 + 0.0002x - 0.0000004\times^2$	0.43
ISL (cm)	10.22	10.81	10.99	10.58	10.82	10.63	0.6772	$Y = 17.67$	–
FSL (cm)	11.48	11.91	13.17	12.11	11.83	11.89	0.8644	$Y = 12.06$	–
GLT (number)	3.66	3.77	3.79	4.05	4.08	4.10	<0.0001	$Y = 3.66 + 0.001x$	0.61
NSL (number)	0.537	0.611	0.669	0.688	0.698	0.877	0.0020	$Y = 0.53 + 0.0006x$	0.29
NFEL (number)	2.01	2.16	2.24	2.39	2.44	2.46	0.0002	$Y = 2.05 + 0.0009x$	0.39

Leaf elongation rate (LER), leaf appearance rate (LAR), phyllochron (PHILO), leaf lifespan (LLS), leaf senescence rate (LSR), stem elongation rate (SER), initial stem length (ISL), final stem length (FSL), green leaves per tiller (GLT), number of senescent leaves (NSL), and number of fully expanded leaves (NFEL) of Massai grass as a function of nitrogen doses.

- Physiological efficiency (PE) measures the efficiency of capture of plant nitrogen capture in biomass yield (Craswell and Godwin 1984; Good, Shrawat, and Muench 2004).
- PE (kg kg^{-1}) = (TFA with fertilization, kg – TFA without fertilization, kg)/(NA with fertilization, kg – NA without fertilization, kg);

The number of rest days of rest was determined by the average interval of days between one cut and the other in the plots. The number of cuts (NC) was determined by the average NC to which the plots were submitted during the experimental period.

Statistical analysis

The assumptions of normality of errors and homoscedasticity of variance were tested using Cramer-von Mises and Brown-Forsythe tests, respectively. The data were submitted to analysis of variance, and when significant, regression analysis was performed. The choice of models was based on the significance (5%) of the linear and quadratic coefficients and the determination coefficient. The regression equations were adjusted based on the treatment averages, with the r^2 obtained through the quotient between the sum of squares of the regression and the total sum of squares. Data were analyzed using R 3.5.1 (R Foundation for Statistical Computing). In the presentation of the results, the estimated values from the generated models were used to infer the increments, reductions, and maximum points of the effects of fertilization on the variables.

The following statistical model was used:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_j$$

where Yij is the value of the experimental unit that received the j-th treatment in the i-th repetition, μ is the effect of the general average, and εj is the effect of the error of the experimental unit that received the j-th treatment in the i-th repetition.

Results

Morphogenic characteristics

LER, LAR, LSR, GLT, NSL, and NFEL increased linearly ($p < 0.05$) with nitrogen fertilization (Table 1). Based on the values estimated by the regression models, the increase in leaf elongation rate was 69.1% at a dose of 500 kg N ha^{-1} compared to the control (no nitrogen fertilization). There was an increase of 214.3% in leaf senescence rate in plants fertilized with 500 kg N ha^{-1}.

Table 2. Morphological composition and height of Massai grass.

Characteristics evaluated	Doses of N (kg ha^{-1}) 0	100	200	300	400	500	*p-value*	Regression Equation	r^2
LB (%)	91.63	94.52	92.23	94.00	91.67	92.00	0.2543	Y = 92.67	–
S (%)	2.02	3.31	3.68	3.75	4.12	4.74	0.0027	Y = 2.45 + 0.004x	0.88
LBS (%)	93.65	97.83	95.91	97.75	95.79	96.74	0.3888	Y = 96.28	–
DT (%)	6.35	2.17	4.09	2.25	4.21	3.26	0.2050	Y = 3.72	–
LSRT	40.36	28.55	25.06	25.06	22.25	19.41	0.0001	Y = 38.23 − 0.04x	0.74
Height (cm)	44.39	44.94	44.04	44.28	42.02	44.16	0.5854	Y = 43.97	–

Leaf blade (LB), stem (S), leaf blade + stem (LBS), dead tissue (DT), and leaf:stem ratio (LSRT) of Massai grass as a function of nitrogen dose.

Increases of 13.7% and 56.6% were observed in the green leaves per tiller and the number of senescent leaves, respectively, at a dose of 500 kg N ha^{-1}. The number of fully expanded leaves increased by 21.95% for the 500 kg N ha^{-1} dose.

Phyllochron and LLS decreased linearly ($p < 0.05$) with nitrogen fertilization. PHILO was 35.8% lower at a dose of 500 kg N ha^{-1} than in the control. The leaf lifespan estimate decreased by 29.0% (recorded in the number of days), and the leaves remained alive. Stem elongation rate was the only morphogenic variable that responded to the quadratic model ($p < 0.05$). The maximum estimated point of 0.050 cm tiller day^{-1} was observed at a dose of 250 kg N ha^{-1}. There was no significant effect ($p > 0.05$) of nitrogen fertilization on the initial stem length and final stem length.

Morphological proportion, biomass accumulation and nitrogen use indices

There was no effect ($p > 0.05$) of nitrogen fertilization on LB, LBS, DT, and height percentages (Table 2). The stalk percentage increased linearly with fertilization ($p < 0.05$). The stalk percentage increase was 81.6% at a dose of 500 kg N ha^{-1} compared to the control. There was a linear reduction in the LSRT with an increase in the nitrogen dose ($p < 0.05$). The leaf:stem ratio decreased by 52.3% when the canopy was fertilized with 500 kg N ha^{-1} compared to the non-fertilized treatment. The canopies showed an average height between treatments of 43.97 cm.

The FAC, DFA, TFA, NUE, LBSA, and LBA responded to the quadratic model ($p < 0.05$) (Table 3). The estimated forage accumulation per cycle values was 2,137 and 2,427 kg DM ha^{-1} for the 0 and 500 kg N ha^{-1} doses, with a maximum of 2,786 kg DM and 287 kg N ha^{-1}, respectively. The estimated daily forage accumulation values were 45.89 and 113.39 kg DM ha^{-1} for the 0 and 500 kg N ha^{-1} doses, with a maximum of 116.03 kg DM with 419 kg N ha^{-1}. For total forage accumulation, the 0 and 500 kg N ha^{-1} doses were 6,906 and 18,186 kg DM ha^{-1}, respectively. The maximum TFA was 19,136 kg DM with 391 kg N ha^{-1}, representing an increase of 177.1% in TFA. In addition, the increase in TFA was 163.3% for the 500 kg N ha^{-1} dose compared to the control.

The NUE responded to the quadratic model ($p < 0.05$), increasing in estimated values from 0.661 to 0.8485 from the 0 to 250 kg N ha^{-1} doses, and later decreased until reaching a value of 0.661 again in the 500 kg N ha^{-1} dose (Table 3). The LBSA increased from 6,544 to 17,684 kg DM ha^{-1}, whereas for LBA the estimated increase was from 6,387 to 16,742 kg DM ha^{-1} for the 0 and 500 kg N ha^{-1}doses, respectively. The maximum point estimated for LBSA was 18,622 kg DM ha^{-1} at a dose equivalent to 391 kg N ha^{-1}. In the LBA, the maximum estimated value was 17,770 kg DM ha^{-1} for 385 kg N ha^{-1}.

Recovery of applied nitrogen was negatively influenced ($p < 0.05$) by the nitrogen doses, where the increase in N doses linearly decreased RAN from 127.6% to 88.8%. Agronomic efficiency and physiological efficiency decreased linearly ($p < 0.05$) by 56.93 and 48.68 kg DM kg^{-1} from N to 22.09 and 24.68 kg DM kg^{-1} from N, applied in doses of 100 to 500 kg N ha^{-1}, respectively.

Table 3. Forage accumulation, number of cuts, and rest period for Massai grass.

	Doses of N (kg ha^{-1})								
Characteristics evaluated	0	100	200	300	400	500	*p-value*	Regression Equation	r^2
FAC (kg DM ha^{-1})	2122	2547	2683	2843	2637	2454	0.0249	$Y = 2136.6 + 4.53x - 0.0079x^2$	0.97
DFA (kg DM ha^{-1})	45.81	77.71	89.86	115.9	112.2	110.7	<0.0001	$Y = 45.89 + 0.335x - 0.0004x^2$	0.97
TFA (kg DM ha^{-1})	6792	12738	15563	19335	18464	18412	<0.0001	$Y = 6905.9 + 62.56x-0.08x^2$	0.98
NUE (kg^2 g $^{-1}$)	0.625	0.859	0.816	0.848	0.742	0.677	<0.0001	$Y = 0.661 + 0.0015x-0.000003x^2$	0.78
RAN (%)	–	126.3	111.2	122.0	96.17	85.32	0.0019	$Y = 137.3 - 0.097x$	0.78
AE (kg kg $^{-1}$)	–	59.46	43.85	41.81	29.18	23.24	<0.0001	$Y = 65.644 - 0.0871x$	0.95
PE (kg kg $^{-1}$)	–	52.31	39.64	34.23	30.02	27.08	<0.0001	$Y = 54.68 - 0.0600x$	0.82
LBSA (kg DM ha^{-1})	6.361	12.462	14.927	18.900	17.686	17.813	<0.0001	$Y = 6544 + 61.78x - 0.079x^2$	0.97
LBA (kg DM ha^{-1})	6.224	12.040	14.354	18.175	16.926	16.940	<0.0001	$Y = 6387.4 + 59.21x-0.077x^2$	0.97
SA (kg DM ha^{-1})	137.2	421.6	572.7	725.1	760.7	872.7	<0.0001	$Y = 235.45 + 1.38x$	0.93
DR (days)	46	33	30	25	24	22	<0.0001	$Y = 40.86 - 0.044x$	0.83
NC (N°)	3.2	5	5.8	6.8	7	7.5	<0.0001	$Y = 3.85 + 0.0081x$	0.91

Forage accumulation per cycle (FAC), daily forage accumulation (DFA), total forage accumulation (TFA), nitrogen utilization efficiency (NUE), recovery of applied nitrogen (RAN), agronomic efficiency (EA), physiological efficiency (PE), leaf blade and stem accumulation (LBSA), leaf blade accumulation (LBA), stem accumulation (SA), number of days of rest (DR), and number of cuts (NC) in Massai grass as a function of increasing nitrogen doses.

Table 4. Chemical composition of Massai grass.

	Doses of N (kg/ha)								
Characteristics evaluated	0	100	200	300	400	500	*p-value*	Regression Equation	r^2
DM (%)	25.59	22.77	21.08	20.88	20.55	19.68	<0.0001	$Y = 24.36–0.01x$	0.69
Ash (%DM)	6.79	6.36	6.27	5.88	5.75	5.68	<0.0001	$Y = 6.68–0.002x$	0.67
OM (%DM)	93.21	93.64	93.73	94.12	94.25	94.32	<0.0001	$Y = 93.32 + 0.002x$	0.66
CP (%DM)	6.83	9.27	11.93	14.28	15.57	17.07	<0.0001	$Y = 7.32 + 0.02x$	0.95
NDF (%DM)	70.78	71.41	72.23	72.86	71.41	71.21	0.5404	$Y = 71.25$	–
ADF (%Dm)	38.02	38.06	38.46	38.71	37.73	37.43	0.2507	$Y = 38.07$	–
HEM (%DM)	38.03	33.35	33.77	34.15	33.98	33.78	0.2348	$Y = 34.51$	–

Dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (HEM) of Massai grass as a function of increasing nitrogen dose.

Stem accumulation increased linearly ($P < 0.05$) with N fertilization. Based on the values estimated by the regression models, the increase in SA was 293.1% at a dose of 500 kg N ha^{-1} compared to the control. The number of days of rest decreased linearly ($p < 0.05$), with values estimated from 40.86 to 18.86 days for doses of 0 to 500 kg N ha^{-1}, respectively. The number of cuts increased ($p < 0.05$) in a linear fashion with values estimated between 3.85 and 7.90 cuts for doses of 0 to 500 kg N ha^{-1}, respectively.

Chemical composition

Dry matter and ash contents decreased linearly ($p < 0.05$) with nitrogen fertilization. There was a reduction in DM and ash of 20.5% and 15.0%, respectively, at a dose of 500 kg N ha^{-1} compared to the control (Table 4). The organic matter and crude protein contents increased linearly ($p < 0.05$) with the nitrogen dose. The increase was 136.6% in the CP content when fertilized with 500 kg N ha^{-1} compared to the canopies without nitrogen fertilization. There was no difference ($p > 0.05$) in the NDF, ADF, and HEM contents of Massai grass.

Discussion

Most of the morphogenic variables related to leaf production showed linear variation with increasing nitrogen dose. A dose of 500 kg N ha^{-1} year^{-1} was found to promote higher rates of leaf appearance and elongation, and a greater number of fully expanded, and green leaves per tiller. Despite allowing a greater proportion and accumulation of stalk, a dose of 500 kg N $ha^{-1} year^{-1}$ increased crude protein content in the forage. However, some variables that correlated with nitrogen utilization efficiency, such as RAN, AE and PE, decreased with the increase in nitrogen dose. In addition, some important variables related to forage accumulation, such as FAC, DFA, and TFA, showed a quadratic correlation. Therefore, the greatest dose of nitrogen does not necessarily promote the greatest accumulation of forage.

Nitrogen fertilization increased protein content but did not influence fiber content

The dry matter and ash content can be explained by the increase in the leaf appearance rate with nitrogen fertilization (Table 1). This caused the collected material to be predominant in young leaves that have a high amount of water compared to older leaves, thus reducing the DM content (Table 4). The organic matter content is a consequence of the increase in protein content, because nitrogen fertilization favors the accumulation of organic compounds in the plant. Several studies have demonstrated an increase in crude protein concentration when pastures are fertilized (Coblentz, Jokela, and Bertram 2014, Coblentz et al. 2017). The crude protein content increased because of the higher presence of free amino acids, which maintain nitrogen in the structure, and of small peptides in the plant tissue in response to the greater nitrogen input in the soil. Therefore, nitrogen absorption increased and was faster than the growth of the plants, as evaluated by the amount of DM produced. The use of nitrogen fertilizers does not always cause changes in the fibrous fraction of the plants (Rogers et al. 1996); therefore, when the pasture is cut at the ideal point and the environmental conditions satisfy the needs of the plant, the changes in components such as neutral detergent fiber, acid detergent fiber, and hemicellulose are minimal. In this assay, there was probably no influence because the harvest was carried out considering 95% light interception.

How does nitrogen fertilization affect the morphological characteristics of Massai grass?

Nitrogen is an element that acts in the growth of tropical grasses and is usually a determining factor in the productive success of this group of plants. The availability of nitrogen in the system directly affects the morphological and genotypic characteristics of forage plants, and the combination of these factors determines the main structural characteristics of pastures (Lemaire and Chapman 1996). In the present study, the positive responses to nitrogen fertilization of Massai grass for leaf elongation rate, leaf appearance rate, and green leaves per tiller are reflections of these processes (Table 1). The leaf elongation rate and leaf appearance rate, this may be related to the high demand and nitrogen deposition in the cell division zones (Gastal and Nelson 1994), which increases the production of cells. These effects were opposite in the phyllochron because this is the inverse of the leaf elongation rate.

The reduction in leaf lifespan with nitrogen doses is explained by the greater tissue renewal in the fertilized plants. Plants with little available nitrogen remain longer with their leaves alive at the expense of expanding new leaves. In the present study, this process was evidenced by the positive responses in appearance and elongation of leaves and, consequently, a reduction in phyllochron in the fertilized plants because there was an increase in the flow of tissues and the proportion of new leaves with high photosynthetic capacity. In a study carried out with another tropical grass, *Urochloa brizantha* ‘Marandu’, Santana et al. (2017) reported that nitrogen fertilization had a negative effect on leaf lifespan, reinforcing the findings of the present study. The authors attributed the potentiation of the leaf lifespan reduction to the growth of the plant during the months of the year with better temperature and precipitation conditions. Therefore, in the climatic conditions of the present study (Figure 1), such a response was reinforced.

The higher the leaf lifespan, the lower the leaf senescence rate. Although we observed an increase in leaf senescence rate with fertilization, the representativeness of the values of this variable was small, which did not cause forage loss in this situation. Such observations can be further reinforced by the lack of a fertilization effect on the percentage of dead tissue (Table 2).

When the pastures intercepted 95% of the incident light, the competition for light between the tillers intensified the stem elongation and leaf appearance rates to place the youngest leaves in the upper extract of the pasture, which increased the stem accumulation and, consequently, the forage mass (Santana et al. (2017). Massai grass, compared to other grasses of the genus *Megathyrsus*, such as Tanzânia grass (Paciullo et al. 2017), has low stem elongation rate, and probably for this reason, the initial stem length and final stem length were not influenced by nitrogen fertilization in the present study, presenting a general average of 17.67 and 12.06 cm, respectively (Table 1). In addition, the use of similar management in all plots, such as a fixed residual height of 15 cm and the cutting condition when the canopy reached 95% light interception, contributed to the similarity between the initial and final stem length treatments.

Decrease in leaf lifespan and increase in green leaves per tiller with nitrogen fertilization probably occurred due to the role of nitrogen in anticipating the senescence process in plants. This can be reinforced by observing the results of the number of senescent leaves and the number of fully expanded leaves, which increased with nitrogen fertilization (Table 1). Therefore, the translocation of nutrients might have assisted in the expansion of new leaves because of the higher growth of the plant (Santana et al. 2017). Thus, a high accumulation of forage is linked to high photosynthetic rates, an increase in respiratory rate, and subsequent tissue senescence. Thus, there is a need to respond to grazing management in Massai grass fertilized with nitrogen, so that the harvest is as efficient as possible, aiming to minimize forage losses during the senescence process.

Although there was an increase in LER and LAR with nitrogen fertilization (Table 1), these variables did not change the leaf blade percentage (Table 2) because the canopy was defoliated at 95% light interception. When evaluating Mombasa grass fertilized with nitrogen doses in humid tropical conditions, Oliveira et al. (2020) also observed similarity in the percentage of LBs, probably due to the height of cut and fixed residue used in the handling of the canopies. This same management technique was probably the main reason for the absence of variation in the percentage of dead tissue percentage. The cut was made under ideal conditions where the apical meristem did not have the opportunity to elongate the stem; therefore, dead tissue accumulation was avoided after the cut. In addition, the high-intensity handling used in the Massai grass in the present study (15 cm residue) might have stimulated the appearance and elongation of new leaves and reduced the proportion of senescent tissues in the material.

The increase in the stem percentage with nitrogen fertilization occurred due to the stimulation of nitrogen in the tissue flow, increasing growth through stem cell division (Dupas et al. 2010; Batista et al. 2014). Although this increase in stem percentage was observed, this change was not as pronounced, as demonstrated by the percentage of LBS (leaf blade + steam) that did not differ with fertilization (Table 2). Therefore, the genetics of Massai grass in an intensive production system responds preferentially to leaf biomass production when environmental conditions are favorable for plant growth (Fernandes et al. 2017).

The reduction in the leaf:steam ratio with nitrogen fertilization occurred because of the increase in the proportion of steam. Therefore, although the cuts were performed under ideal conditions, fertilization stimulated stem growth and development. This portion is not recommended for consumption by the animal, because the leaf blade portion is the most preferable for the animal, as it presents better nutritional qualities than the culm portion (Drescher et al. 2006). Even with the increase in the percentage of stems and reduction in the leaf:steam ratio with nitrogen fertilization, all canopies maintained a leaf blade percentage above 90%. This provided canopies with a large percentage of leaves, which is interesting from the point of view of grazing, given that the animals prefer the consumption of leaves. The absence of a difference in canopy height was due to the use of the same light interception as a criterion for harvesting forage in all the plots. The height of 44 cm, below that observed in the literature (Veras et al. 2020), can be explained by the plasticity of the canopy structure under the conditions of the present study, mainly because the Massai grass was managed with high defoliation intensity.

The quadratic relationship between biomass accumulation and nitrogen fertilization

LAR and LER increased as nitrogen fertilization increased; therefore, Massai grass pastures reached the forage harvest point more quickly. This caused the interval between cuts to be reduced, and the rest period necessary for the plant to reach the ideal harvest point. Therefore, the number of cuts was higher with nitrogen fertilization (Table 3), which increased the total forage accumulation in treatments with more cycles. Similarly, when evaluating Mombasa grass fertilized with nitrogen doses in tropical humid conditions, Oliveira et al. (2020) observed an increase in the number of grass cuts. This would produce a need for shorter grazing cycles in intermittent stocking systems. However, if such responses are not made in practice, forage may be wasted in areas fertilized with nitrogen.

The forage accumulation per cycle, daily forage accumulation, and total forage accumulation results (Table 3) can be explained by the increase in LER and LAR with nitrogen fertilization (Table 2). In addition, forage accumulation was influenced by the leaf blade percentage, which was the component that had the highest participation in the total biomass of the pasture. However, this biomass was composed of new leaves with low dry matter content (Table 4), which contributed to the decrease in the FAC when Massai grass was fertilized with amounts above 287 kg N ha^{-1}, resulting in a quadratic adjustment in this variable. FAC, DFA, and TFA increase the stocking rate of pastures because they depend on leaf blade production and the appearance of new leaves. Therefore, these are reflections of the fertility and ability of forage plants to use soil nutrients efficiently (Bueno et al. 2021).

The quadratic response in the FAC, DFA, and TFA can be explained by the high nitrogen doses, close to the maximum points estimated by the equations. Such doses were applied only during the rainy season, corresponding to approximately six months. In addition to the greater biomass contribution from young leaves with low dry matter levels, reductions in accumulation might be related to mutual shading caused by an increase in leaf area in response to nitrogen fertilization. Such facts can be corroborated with by leaf blade + stem accumulation and leaf blade accumulation, which had the same quadratic response of the variables previously presented. Although an increase in stem accumulation was observed with nitrogen fertilization, it had a low proportion when TFA was observed. The forage accumulation observed in the present study clearly demonstrated the effect of nitrogen fertilization, up to a certain dose, on the production of forage mass and, consequently, the possibility of increasing the carrying capacity of tropical pastures fertilized with nitrogen (Delevatti et al. 2019).

Forage accumulation is the most important variable influencing animal production. Support capacity of a pasture area is calculated using forage accumulation data. Among the evaluated accumulations measurements, TFA is of fundamental importance for planning, as it represents the total amount of forage produced per production period. From the quadratic regression model (Table 3), the nitrogen dose that promoted the highest TFA was 391 kg N ha^{-1} $year^{-1}$.

Nitrogen fertilization considerably increases the accumulation of forage in the pastures of *M. maximus* 'Mombasa' (Silva et al. 2020). The authors explained this effect by the fact that nitrogen fertilization accelerates growth, tillering, leaf production, and the expansion of the aerial part and root system. Several studies have reported a positive effect of nitrogen fertilization on pastures (Dupas et al. 2010; Batista et al. 2014). However, Fernandes et al. (2015) suggested that it is possible that the soil can supply part of the nitrogen needs of forage grasses, depending on the type of soil and management of the area, reaching a reasonable production level.

Nitrogen use: what dose is recommended?

The efficiency of nitrogen use in food production worldwide is very low, and nitrogen recovery from fertilizers can vary among crops, management practices, soil properties, environmental conditions, and the nitrogen source used (Espindula et al. 2010). Thus, maximizing the efficiency of converting nitrogen from fertilizers to forage dry matter is extremely important for the final bioeconomic results of nitrogen fertilization in pastures (Martha Júnior, Alves, and Contini 2012). In the present study, the nitrogen utilization efficiency quadratic response followed the TFA response (Table 3), as was is a dependent variable.

The lower RAN with nitrogen fertilization was expected due to the higher dry matter yield, nutrient concentration, and extraction of nitrogen by the plants. In addition, at higher doses, there was a greater chance of nutrient loss through volatilization, leaching, denitrification, and surface washing. Another aspect that possibly contributed to the reduction of RAN with nitrogen fertilization is that in the present study, as the sampling was made only of leaves and stems above 15 cm, there was no evaluation of the nitrogen accumulated in the roots and residues of the aerial part (below 15 cm), which might have influenced the RAN.

Nitrogen recovery can be over 80% in tropical grasses, indicating that the fertilizer has been applied properly. RAN values can reach high values and exceed 100% (Rowlings et al. 2016). The results showed that nitrogen fertilization affected the characteristics of the pasture and the agronomic efficiency of applied nitrogen (Table 3). The nitrogen requirements of the plant were possibly met, negatively influencing the RAN (Galindo et al. 2017). Primavesi et al. (2006), working with nitrogen doses with ammonium nitrate and urea sources in Marandu grass, found that the RAN varied with the sources and doses of N, showing that with increasing in the doses of N, there was a decrease in the recovery of nitrogen for both sources. The decrease in AE with nitrogen fertilization indicates that there was probably less use of nitrogen by plants. This usually occurs due to the loss of this nutrient because of leaching or volatilization at higher doses, caused by the high rainfall characteristic of the region and that occurred during the experimental period. The results also corroborate those of Galindo et al. (2017), who observed better EA at lower doses of nitrogen.

Regarding physiological efficiency, the decrease with nitrogen fertilization was similar to that observed by Galindo et al. (2017), who evaluated Marandu grass pastures. The authors observed that the sources did not influence the PE and reported a significant difference in doses and years of pasture recovery in the PE, where during all recovery years, there was a linear reduction of PE with an increase in the applied nitrogen doses. The highest PE values were observed at doses of 100 kg ha^{-1} $year^{-1}$ and the lowest at the maximum dose in all the years evaluated.

Regardless of the dose that potentiated the TFA, NUE, EA, and chemical composition of the forage, the results shown in the present study are extremely important for application in practical situations, as they allow the use of nitrogen fertilization at different technological levels. A dose of 391 kg N ha^{-1} $year^{-1}$ promoted the highest total forage accumulation (representing all forage produced during a forage utilization period). A dose of 287 kg N ha^{-1} $year^{-1}$ promoted the highest nitrogen use efficiency (NUE) during our experimental evaluations. Therefore, it is necessary to establish production goals, and design fertilization to fit forage budgets.

Conclusion

Increasing doses of nitrogen favor the productivity and nutritional quality of Massai grass. Nitrogen fertilization demands special attention, because while it stimulates plant production, it is also linked to the efficient use of nitrogen, with reduced losses and mitigation of environmental impacts. The accumulation of total forage of the cultivar reached maximum production with an estimated application of 391 kg N ha^{-1} $year^{-1}$, demonstrating the potential of this management practice in humid tropical conditions.

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