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Effect of Nitrogen Fertilization on Production, Chemical Composition and Morphogenesis of Guinea Grass in the Humid Tropics

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Abstract: The use of nitrogen fertilization in tropical grasslands is a strategy that may reduce the pressure of livestock production on biome areas with humid forests. The objective of this study was to assess the use of different doses of nitrogen in Guinea grass (*Megathyrsus maximus* (Jacq.) con. Mombasa) cultivated in the humid tropics. Doses of 0, 10, 20, 30, 40, and 50 kg of N ha^{-1} $application^{-1}$ were applied during two consecutive years in experimental plots. The experimental area is located in the northeast of Pará, Brazil, with a predominantly tropical climate according to the Köppen classification. The morphogenic, production, and qualitative characteristics of the forage were evaluated. The morphogenic variables, rate of leaf appearance and rate of leaf elongation, in addition to daily accumulation of forage and crude protein content, increased with increasing doses of nitrogen. The morphological structure of the pasture was not modified. The number of cuts increased, whereas the number of days of recovery decreased, in the rainy season with increasing doses of nitrogen. The use of nitrogen fertilization does not alter the structure of the pasture; however, it improves the morphogenic, production, and chemical characteristics of Guinea grass.

Keywords: Af climate; crop-livestock; dry season; fertilizer; rainy season

1. Introduction

Grasslands are among the most widely distributed terrestrial ecosystems in the world [1]. The low productivity of some of these areas, especially in tropical regions, has often limited the competitiveness of agricultural activities. In some cases, this situation creates pressure to expand grasslands and, consequently, livestock production in areas of native vegetation [2]. Intensification of these areas is an option to reverse this situation [3]. Strategies of grassland-use intensification are expected to increase further, especially in humid and sub-humid environments [4], where soil humidity is not a limiting factor for most of the year [5].

One means of intensifying livestock production in grasslands is by fertilizing pastures with nitrogen (N) and optimizing the proportion of forage consumed by animals through grazing management [6]. Nitrogen (N) fertilization increases the carrying capacity (i.e., number of animals per area) of pastures [7].

Therefore, to maximize biomass production it is important to maintain the concentration of N in the soil during the growth season, which requires optimal supply of this nutrient to the soil [8].

The results from previous studies indicate that N stability is highly sensitive to variations in soil moisture [9]. Under humid tropical climate conditions, rainfall suffices to meet the water demand of forage plants for most of the year, and the availability of light and temperatures around 30 °C definitively contribute to increasing the yield of C4 forage plants, such as Guinea grass [5,10].

Unsuccessful performance of tropical grasses may be mainly attributable to errors in management, in addition to the edaphoclimatic variables [11]. Thus, these errors are probably amplified in a humid tropical climate.

Therefore, the objective of this study was to evaluate the morphogenic and botanical characteristics, yield, and quality of forage following application of increasing doses of N fertilizer throughout the year under the climatic conditions of the humid tropics.

The present study tested the hypothesis that increasing doses of N fertilizer in the humid tropics increases the production and quality of forage, changes grass morphogenic and botanical characteristics, and reduces production seasonality throughout the year.

2. Materials and Methods

2.1. Experimental Site and Climatological Data

This trial was conducted in the municipality of Castanhal, Pará, Brazil, located at the geographic coordinates 1°18′17.2″ latitude (S) and 47°56′30.2″ longitude (W).

The experimental area is located in the northeast of Pará, where the predominant climate is Af (tropical climate without dry season) according to the Köppen classification, because it has high temperatures with small thermal amplitudes, abundant precipitation, and relative humidity between 85 and 95%. There is one season of nine months with higher rainfall and another of three months with lower rainfall [12] (Figure 1).

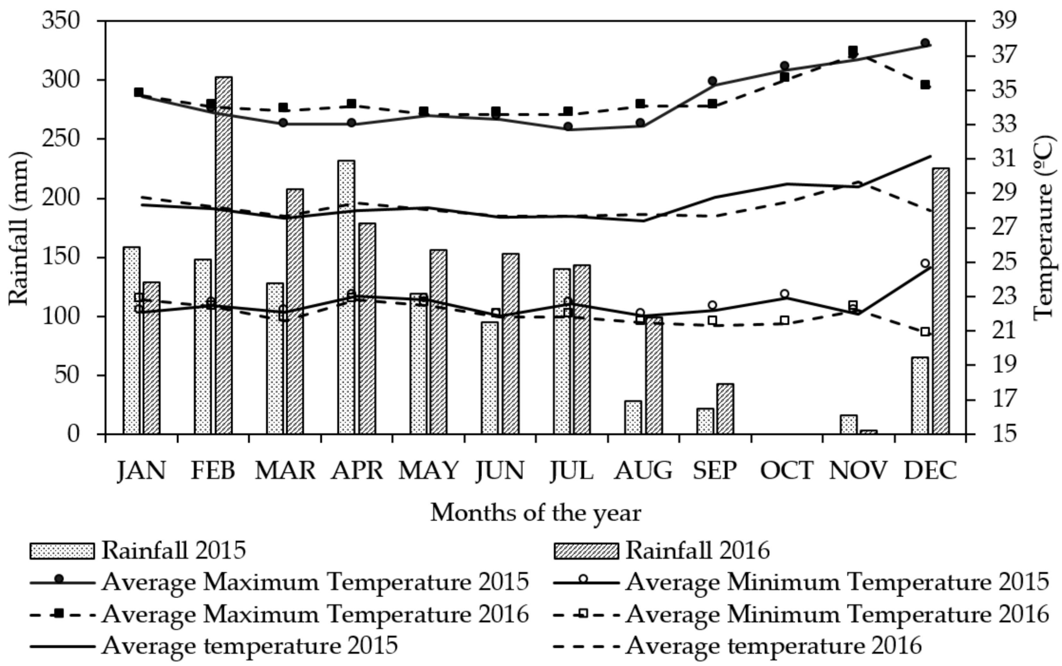

Figure 1. Monthly total rainfall and average temperatures during the experimental period.

The soil is classified as Udox (suborder of Oxisols) with texture sandy Loam, according to the U.S. Soil Taxonomy compilation [13]; the chemical composition of the soil is shown in Table 1. The soil acidity of the experimental area was corrected two years before the experiment started on 15 November

2013 with 1000 kg of dolomitic limestone. Fertilization was performed with 129 kg ha^{-1} of P_2O_5 in the form of monoammonium phosphate and with 215 kg ha^{-1} of K_2O in the form of potassium chloride. According to the soil analysis the application of these fertilizers was conducted in conjunction with the planting of forage.

Table 1. Analytical results of soil analyses performed at the experimental site.

pH	Ca	Mg	Al	H + Al	CEC	K	P	OM	BS%	Clay	Silt	Sand
			cmolc dm^{-3}				mg dm^{-3}	g dm^{-3}			g kg^{-1}	
						2013						
4.7	1.2	0.3	0.2	3.0	4.59	80	0.6	16	35	168	56	776
						2014						
4.9	1.8	0.6	0	2.4	4.87	50	1.8	19	51	145	127	728
						2015						
5.1	2.1	0.9	0	2.7	5.85	110	4	28	54	94	119	787

pH, hydrogen potential; Ca, calcium; Mg, magnesium; Al, aluminum; H + Al, hydrogen plus aluminum; CEC, cation exchange capacity; K, potassium; P, phosphorus; OM, organic matter; BS%, percentage base saturation.

Guinea grass (*Megathyrsus maximus* (Jacq.) con. Mombasa) was sown in the experimental plots in January 2014 at 8.6 kg ha^{-1} of seeds broadcast directly in beds measuring 12 m^2 (4 × 3 m), separated by corridors approximately 1 m wide. Subsequently, growth in the beds was periodically monitored throughout 2014. When the canopy reached a height of 90 cm, it was cut mechanically with an electric pruner (pruner HS 45) at a residual height of 40 cm above the ground.

Soil was analyzed on 22 October 2014, and the experimental areas were limed with 500 kg ha^{-1} of dolomitic limestone in November 2014. Phosphate fertilizer with 83 kg ha^{-1} of P_2O_5 in the form of monoammonium phosphate was applied together with the first N fertilizer of the experimental period on January 7, 2015. Potassium fertilization was divided into four annual applications of 60 kg ha^{-1} K_2O; the first application occurred in January at the beginning of the experiment and the others in April, July, and October, totaling 240 kg ha^{-1} year^{-1} of K_2O in the form of potassium chloride. The same fertilization procedure was performed with limestone, phosphorus, potassium, and N in the second experimental year.

The experiment data collection was initiated in 2015 and lasted for 2 years, with the first year from January 2015 to January 2016 and the 2nd year from January 2016 to February 2017.

2.2. Treatments and Experimental Design

A randomized complete block design was used with six treatments corresponding to doses of 0, 10, 20, 30, 40, and 50 kg of N ha^{-1} application^{-1}, which were applied after each cut. The source of the fertilizer used was agricultural urea, comprising 45% of the N. There were four repetitions for each treatment, yielding a total of 24 experimental units (plots). The evaluations spanned two complete years, including two rainy seasons and two dry seasons.

2.3. Plot Management

Canopy height of Guinea grass (*Megathyrsus maximus* (Jacq.) con. Mombasa) was measured with a rod in centimeters, with the average height of the canopy calculated using 10 points within each plot. When the plots reached an average height of 90 cm, they were cut at the residual height of 40 cm. The cuts were made with the forage in the vegetative phase.

The cuts were made when the plot reached a height of 90 cm, which corresponds to the point when the canopy intersects 95% of the solar radiation on the canopy, because at this time the plant has a higher proportion of leaves, and lesser stems and dead parts, which provides the best relationship between quality and productivity [14].

2.4. Analyses of Forage Morphogenic Characteristics

Ten tillers were marked per experimental plot by choosing the group of tillers in which the vegetation condition was representative of the average condition of the bed. The tillers were identified with numbered plastic wires, and the tillers were evaluated every two days.

In the tiller evaluations, the leaves were classified as fully expanded (presenting fully exposed and visible ligule), expanding (without a visible ligule), and senescent (when the leaf blade showed some sign of senescence (yellowing)). Leaves in which more than 50% of the blade length was compromised by senescence were considered dead. The stem length was measured from ground level to the last fully expanded leaf. The measurements were made with graduated rulers and the data were recorded in previously prepared spreadsheets.

The evaluations were performed throughout the two years and after each cut of the tillers; after each evaluation, they were replaced by new tillers.

From the tiller evaluations, it was possible to calculate the following morphogenic and structural variables: leaf appearance rate (LApR; leaves tiller^{-1} day^{-1})—number of leaves appearing per tiller divided by number of days of the evaluation period; leaf elongation rate (LElR; cm tiller^{-1} day^{-1})—sum of all the leaf blades elongation per tiller divided by number of days of the evaluation; stem elongation rate (stem + pseudostem; SElR; cm tiller^{-1} day^{-1})—variation in stem length per tiller divided by number of days of the evaluation; phyllochron (PHYLLO; days leaf^{-1} tiller^{-1})—calculated by the inverse of the LApR; leaf senescence rate (LSR; cm tiller^{-1} day^{-1})—sum of the senesced lengths of leaf blades per tiller divided by number of days of the evaluation period; stem growth (SG; cm)—measured above the cutting height until the last expanded leaf in the tiller; number of senescent leaves (NSL; number)—average number of leaves with more than 50% of the leaf blade in a state of senescence per tiller; number of expanding leaves (NEL; number)—average number of leaf in expansion per tiller; number of live leaves per tiller (NLL; number)—average number of expanding leaves and leaves expanded per tiller, not considering the leaves with more than 50% of this length senesced; number of mature leaves (NML; number)—average number of mature leaves per tiller; leaf life duration (LLD; days)—period of time between the leaf appearance and its death, estimated by the multiplication of the number of live leaves per tiller by the inverse of leaf appearance rate [15]; initial and final stem length (ISL and FSL; cm)—distance between the ligule of the last expanded leaf in relation to the soil.

2.5. Analyses of Forage Production and Botanical Characteristics

To estimate forage accumulation per hectare, one forage sample was collected per plot at the end of each regrowth cycle. The forage was cut and collected from within a 1.0 × 0.5 m metal frame, 40 cm above ground level. Soon after collection, the samples were placed in plastic bags, identified, and weighed.

A subsample of approximately 300 g was taken to determine the percentage of dry matter (DM) after drying in a forced ventilation oven at 55 °C for approximately 72 h. Another subsample of approximately 500 g was separated into leaf (LB %), stem (STEM %), and senescent material (SEN %). After separation the parts were dried in a forced ventilation oven at 55 °C for approximately 72 h and the quantities found were used to calculate the percentages of the parts. The leaf:stem ratio (LB:STEM) was calculated using the ratio between the amount of leaves and the amount of stem.

To estimate daily forage accumulation (DA, kg DM ha^{-1} day^{-1}), the forage accumulation per cycle was divided by the number of regrowth days. The regrowth period (RP, days) was calculated considering the average interval in days between two subsequent cuts in the plot. The number of cuts (NC, number) was calculated by the average number of cuts that the plot had during the evaluation period.

2.6. Analyses of Forage Chemical Characteristics

Samples of forage were collected and pre-dried at 55 ± 5 °C, until constant weight, and then ground in a Wiley mill with a 1 mm mesh sieve. The following chemical analyses were performed: dry matter (DM); mineral matter (MM) (method 942.05; AOAC 1995); crude protein (CP) (method 984.13; AOAC 1995); organic matter (OM), calculated as 100 minus the percentage of MM [16]; neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents determined according to the sequential method without sodium sulfite [17]; cellulose was solubilized using sulfuric acid at 72%, and the remaining residue corresponded to the amount of lignin in the sample [18]. The ethereal extract (EE) (method 920.39, AOAC 1995) [16] was analyzed using ANKOM equipment and the procedures outlined by ANKOM Technology Corp. (Fairport, NY, USA).

2.7. Statistical Analyses

The data were analyzed using the SAS PROC MIXED procedure [19], with the plots considered as experimental units. The doses of N fertilizer were considered as fixed effects and the blocks and their interactions as random effects. Measurements were repeated over time because the evaluations were performed in successive cycles to verify the best covariance structure for each variable, which was analyzed by the Akaike test. Polynomial orthogonal contrasts were used to determine the nature of the responses following application of the fertilizer doses, which could be first degree (linear), second degree (quadratic), third degree (cubic), or fourth degree (quartic), and the contrast of the rainy season against the dry season, whereby the rainy season was from January to September and the dry season was from October to December (Figure 1). September and December were allocated according to forage behavior in relation to climate because their results were influenced by the weather of the previous months. The results were considered significant when $p < 0.05$.

3. Results

None of the variables studied presented third- or fourth-degree effects; therefore, only the *p*-values for linear and quadratic effects are described herein.

3.1. Morphogenic Characteristics

Morphogenetic and structural variables LElR, LApR, NLL, FSL, SElR, and SG presented increasing linear responses ($p < 0.05$) with increasing N doses. PHYLLO presented an inverse result ($p < 0.05$) compared with LApR, in addition to LLD, LSR, and NSL, which tended to decrease. The variables NEL, NML, and ISL presented a quadratic effect ($p < 0.05$) (Table 2).

When the means of the morphogenetic and structural variables were compared between the rainy and dry seasons, there were no differences for NEL, NML, NLL, LSR, NSL, and ISL ($p > 0.05$) (Table 2). LLD and PHYLLO were lower in the rainy season compared to the dry season, and LElR LApR, FSL, SElR, and SG were higher in the rainy season compared to the dry season ($p < 0.05$) (Table 2).

Table 2. Morphogenic characteristics of Guinea grass.

Variable	kg N ha^{-1} Application^{-1}							Season			p-Value [1]		
	0	10	20	30	40	50	SEM	Rainy	Dry	SEM	L	Q	Season
LElR (cm tiller^{-1} day^{-1})	3.98	4.49	5.37	5.77	5.78	6.13	0.30	6.20	2.91	0.26	<0.01	0.15	<0.01
LApR (leaves tiller^{-1} day^{-1})	0.06	0.07	0.08	0.09	0.09	0.10	0.00	0.10	0.05	0.00	<0.01	0.14	<0.01
PHYLLO (days leaf^{-1} tiller^{-1})	15.6	15.8	13.7	12.7	12.6	11.8	0.62	11.2	20.2	0.56	<0.01	0.80	<0.01
NEL (number)	0.92	0.99	1.01	0.99	0.96	0.97	0.05	1.01	0.91	0.05	0.03	<0.01	0.73
NML (number)	3.05	3.32	3.55	3.52	3.51	3.56	0.10	3.59	3.02	0.09	<0.01	0.02	0.12
NLL (number)	4.35	4.42	4.64	4.55	4.62	4.69	0.09	4.70	4.18	0.09	<0.01	0.25	0.03
LLD (days)	70	67	60	56	56	53	2.60	52	82	2.40	<0.01	0.32	<0.01
LSR (cm tiller^{-1} day^{-1})	0.36	0.26	0.20	0.25	0.09	0.27	0.08	0.25	0.22	0.08	<0.01	0.96	0.21
NSL (number)	0.38	0.23	0.24	0.25	0.18	0.30	0.13	0.27	0.27	0.13	<0.01	0.20	0.23
ISL (cm)	28.2	29.4	30.6	30.2	29.9	30.1	0.84	29.4	30.8	0.80	0.02	0.04	0.50
FSL (cm)	34.3	35.8	37.1	37.4	37.7	39.2	2.18	37.5	35.5	2.21	<0.01	0.20	<0.01
SElR (cm tiller^{-1} day^{-1})	0.21	0.26	0.27	0.30	0.30	0.39	0.07	0.34	0.16	0.07	0.01	0.45	<0.01
SG (cm)	5.61	6.34	6.58	6.88	7.49	9.06	1.75	7.82	4.95	1.76	0.01	0.93	<0.01

[1] Probability associated with the F test for contrasts; LElR, leaf elongation rate; LApR, leaf appearance rate; PHYLLO, phyllochron; NEL, number of expanding leaves; NML, number of mature leaves; NLL, number of live leaves; LLD, leaf life duration; LSR, leaf senescence rate; NSL, number of senescent leaves; ISL, initial stem length; FSL, final stem length; SElR, stem elongation rate; SG, stem growth; SEM, standard error of the mean; L, linear contrast; Q, quadratic contrast.

3.2. Productive, Strucutural and Botanical Characteristics

Increasing the N dose promoted quadratic effects in the DA, STEM %, NC, and RD ($p < 0.05$), but did not significantly change the leaf blade percentage (LB %) or leaf blade/stem ratio (LB:STEM) ($p > 0.05$). There was a linear decrease in senescent material percentage (SEN %) ($p < 0.05$) with increasing N doses (Table 3).

The comparison between rainy and dry seasons revealed no significant difference in the LB % or the LB:STEM ratio ($p > 0.05$) (Table 3). In the rainy season, the percentage of DA, STEM, SEN, and NC were higher than in the dry season (Table 3). The number of RP was lower in the rainy season compared with the dry season (Table 3).

3.3. Chemical Characteristics

There was a significant effect ($p < 0.05$) for all variables relating to forage chemical composition, except EE and ADF in case of linear contrast (Table 4). As the N dose increased, a decreasing linear effect was observed only for DM, whereas the mean values for OM, CP, NDF, hemicellulose (HEM), and lignin (LIG) linearly increased. MM decreased and fitted quadratically, with average values ranging from 6.44 to 7.37 (Table 4).

When the influence of season on the forage chemical composition was evaluated, a significant effect was observed for DM, OM, MM, and CP (Table 4) ($p < 0.05$) with no effect of season for EE, NDF, ADF, HEM, and LIG (Table 4) ($p > 0.05$). In the rainy season, the average DM and OM contents were lower compared with those in the dry season; the opposite effect was observed for MM and CP, which were higher in the rainy season.

Table 3. Productive and botanical characteristics of Guinea grass.

Variable	kg N ha^{-1} Application^{-1}							Season			*p*-Value [(1)]		
	0	10	20	30	40	50	SEM	Rainy	Dry	SEM	L	Q	Season
DA (kg DM ha^{-1} day^{-1})	52.4	73.7	83.4	84.2	87.3	98.3	5.99	91.1	52.1	5.75	<0.01	<0.01	<0.01
LB (%)	87.3	86.9	86.4	86.0	86.9	86.2	2.64	88.7	85.1	2.64	0.61	0.72	0.24
STEM (%)	5.27	5.87	6.69	7.14	6.68	7.27	0.57	6.91	5.74	0.60	<0.01	0.04	0.02
SEN (%)	7.33	7.16	6.90	6.79	6.32	6.43	0.15	7.18	6.22	0.12	<0.01	0.18	<0.01
LB:STEM	15.7	15.7	14.3	14.2	15.1	15.3	1.37	14.5	14.9	1.33	0.37	0.37	0,67
RP (days)	40	34	33	33	33	32	1.75	28	51	1.70	<0.01	<0.01	0.04
NC (number)	7	9	9	10	10	10	0.35	8	2	0.39	<0.01	<0.01	0.03

[(1)] Probability associated with the F test for contrasts; DA, daily forage accumulation; LB, leaf blade; STEM, stem; SEN, senescent material; LB: STEM, leaf:stem ratio; RP, regrowth period; NC, number of cuts; SEM, standard error of the mean; L, linear contrast; Q, quadratic contrast.

Table 4. Chemical composition of Guinea grass.

Variable	kg N ha^{-1} Application^{-1}							Season			*p*-Value [(1)]		
	0	10	20	30	40	50	SEM	Rainy	Dry	SEM	L	Q	Season
DM (g kg^{-1})	264.2	251.8	248.5	242.3	245.1	239.1	11.20	233.3	287.6	11.13	<0.01	0.06	0.01
OM (g kg^{-1} DM)	925.4	927.9	929.5	930.8	935.2	935.6	1.63	928.4	937.6	1.28	<0.01	0.27	<0.01
MM (g kg^{-1} DM)	72.3	72.5	73.7	69.1	64.5	64.4	2.08	73.0	60.1	1.72	<0.01	0.03	<0.01
EE (g kg^{-1} DM)	19.6	19.9	20.6	20.3	21.7	20.7	0.97	20.4	22.8	1.11	0.10	0.59	0.68
CP (g kg^{-1} DM)	87.7	101.9	107.3	117.6	121.2	136.6	5.31	121.5	89.6	4.54	<0.01	0.34	<0.01
NDF (g kg^{-1} DM)	648.5	667.1	668.8	662.4	681.2	669.5	11.53	670.1	657.3	11.98	<0.01	0.77	0.50
ADF (g kg^{-1} DM)	350.6	360.3	355.5	351.7	358.4	357.2	3.34	356.0	353.5	4.10	0.57	0.73	0.56
HEM (g kg^{-1} DM)	309.7	310.4	312.8	314.2	324.4	319.9	6.80	316.1	314.0	0.70	<0.01	0.14	0.13
LIG (g kg^{-1} DM)	24.3	27.1	27.1	25.6	28.0	27.5	0.91	27.0	2.17	1.01	0.04	0.51	0.29

[(1)] Probability associated with the F test for contrasts; DM, dry matter; OM, organic matter; MM, mineral matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber ADF; HEM, hemicellulose; LIG, lignin; SEM, standard error of the mean; L, linear contrast; Q, quadratic contrast.

4. Discussion

4.1. Morphogenic Characteristics

There is a dependent relationship between LElR, LApR, and PHYLLO, because LElR acts as the main modifier of LApR, and in response to N fertilization, leaf elongation is accelerated and the interval between the appearance of new leaves is shorter [20], decreasing PHYLLO (Table 2). LApR was directly related to final leaf size and tiller number; the results of PHYLLO were inverse, with N decreasing the period between the emergence of new leaves.

Increasing doses of N have an effect on the apical meristem of the plants and is the factor that most contributes to the increase in the number of dividing cells and to leaf elongation [21]. The PHYLLO reduction and LElR increase occurred due to the ideal conditions of temperature and nutrient supply [20,22]. N available to the plants promotes the development of new tissues [22]; these physiological events induce the production of hormone metabolites, such as auxins and gibberellins, that are associated with the growth of plant tissues [23].

The values of the morphogenic variables LElR, LApR, PHYLLO, FSL, SElR, and SG (Table 2), which were directly related to the DA (Table 3), were higher in the rainy season than in the dry season. Zanine et al. [24], in a study carried out in a Cwa (dry winter and hot summer) climate according to the Köppen classification, comparing summer (rainy season) with winter and the beginning of spring (dry season), found LElR, LApR, and SElR values to be 171%, 168%, and 519% higher in summer, respectively, compared to winter and beginning spring. Barbosa et al. [25] evaluated Guinea grass carried out in an Aw (tropical climate with dry winter) climate according to the Köppen classification, and found values of LElR, LApR, and SElR were 251%, 125%, and 625% higher, respectively, in summer compared to winter. In our study, these differences were only 113%, 100%, and 113%, respectively, in a comparison of rainy and dry seasons for LElR, LApR and SElR. This demonstrates that the magnitude is lower in the Af climate compared with similar experiments conducted in locations with different climate types.

The responses of NEL and NML indicated that, even when there was a positive response to fertilizer application, this response was limited due to the frequency of defoliation promoted by N with acceleration of tissue growth (Table 2). At this time, many leaves are pruned in the upper strata and the tillers may be in different morphological stages.

NLL (Table 2) results from LLD and is therefore characterized as a stable genotypic trait when adequate nutritional support is provided. Previous studies investigating N fertilization have also reported an increase in NLL [20,26]. Stress conditions may decrease the number of live leaves. In our study, NLL decreases in the dry season compared to the rainy season (Table 2); this reduction was caused by water stress. The reduction in LLD (Table 2) because of increasing N dose is explained by the high rate of tissue renewal; the absence of N induces senescence and the longevity of leaves rather than the appearance of new leaves [22,27]. Thus, this morphogenic variable determines the balance between growth and senescence.

There were no significant differences in NEL and NML between the rainy and dry seasons, whereas LLD was higher in the dry season (Table 2). This was because water influences the physiology and morphology of the plants and its absence may compromise production, leaving the plants in a state of water stress. Thus, in this trial, even with nitrogen availability, sub-optimal water conditions increase LLD.

The plots were cut when they reached their productive peak (90 cm) because this was the management strategy used in the present study; thus, water increased the number of live leaves and shortened their lifespan. Under these conditions, biomass production and the number of cuts increased to avoid senescence losses (Table 3). These results reflect the importance of using N fertilization, especially when associated with favorable rainfall and temperature conditions.

The decreased senescence (LSR and NSL) (Table 2) indicates that the increased number of cuts in the treatments with higher fertilizer doses affected the residual leaf area; therefore, fewer leaves entered senescence as the dose increased. The low LSR in plants that received N contributes to forage quality and probably to increased tillering.

Fertilization promoted an increase in STEM, ISL, FSL, SG, and SElR (Table 2). An increase in the stem may be somewhat undesirable because it decreases the leaf/stem ratio, which affects grazing efficiency. However, in our study, the plant structures related to leaves also increased (Table 2). As a result, the LB:STEM ratio (Table 3) was not altered and therefore did not affect the canopy structure.

The FSL, SElR, and SG variables decreased with lower rainfall, and naturally larger stem portions were observed under better water conditions (Table 2).

Stem accumulation occurs at the end of productive development or at the beginning of the reproductive process and flowering, resulting in larger portions of stem. Both of these factors are accelerated by climate and temperature [28].

4.2. Productive, Structural, and Botanical Characteristics

The DA of the forage increased with increasing N doses (Table 3) because this nutrient increased tissue flow mainly in areas of intense cell division. The use of N fertilization increases the availability of N in the soil, forage yield, and dry matter production [29]. Better DA results probably translate into an increase in animal stocking rates [7].

However, nitrogen fertilizers must be used carefully. The quadratic effect of N on DA (Table 3) shows a reduction in the amplitude of the response to N application from the dose of 20 kg N ha^{-1} $application^{-1}$; this indicates that from that dose the gain in dry matter production per kg of N applied is likely to be reduced. Furthermore, by raising the N dose, N losses increase due to leaching and volatilization [30], and greater greenhouse gas production can result [31]. Another important factor that can increase N losses is the presence of moisture in the soil [30], a situation similar to that of this study. Nonetheless, pastures professionally managed and fertilized with N have high potential to sequester CO_2 [32].

During the dry season, the forage DA was 58% of that found in the rainy season (Table 3). This shows that there was much less seasonality in the annual forage production than reported in studies performed in other climates, which have concentrated on approximately 80% of the production occurring in the rainy season and 20% in the dry season [33], and vary according to the level of intensification adopted for grazing management. Hofer et al. [8] suggest that when the forage plants are not under extreme water stress, fertilization with N may partly mitigate the loss of biomass.

Thus, when considering the management of grazing under the conditions prevalent in the present study, we can maintain distinct management strategies from those of other regions. This is because the smaller difference in production between seasons makes strategies such as deferred grazing and animal supplementation in the dry season successful, allowing high gains per animal and per area.

The increased N dose did not change the LB % (Table 3), which indicates that the participation of this anatomical component in the plant structure is most related to the forage management, such as entry and exit height, rather than the applied fertilization.

The stem percentage increased with increasing fertilizer dose. In this case, the stem increase was due to the capacity of N to maintain plant productivity, which is responsible for plant size and, consequently, the stem size, appearance, and development of the tiller.

The SEN % (Table 3) decreased because N application increases the production of cytokine hormones [23], which inhibit the senescence of plant organs. In the apical zones of root growth in plants, these hormones are transported by the xylem and distributed by transpiration and redirection mainly to mature leaves, inhibiting senescence and keeping the leaves alive. Even with the increased stem fraction observed following treatment, the LB:STEM ratio was not significant. Thus, this indicates that even an increase in the stem was not sufficient to induce changes in this relationship when the leaf fraction was predominant throughout the experimental period.

The results of this study indicate that the plant structure was more influenced by the imposed management than by N fertilization. The practical implication of this finding is that management of the forage with respect to its physiology will enable the same plant structure to develop regardless of the applied N dose; however, the importance of the fertilization translates into a higher number of cutting cycles and shorter regrowth period.

The reduction in RD and the consequent increase in NC (Table 3) was due to the speed of reactions induced by N in the plant, accelerating cell multiplication processes, mainly in the leaves [34]. The results of RD and NC show that the use of N fertilization promotes more efficient systems; intensification promoted by fertilization improves grassland systems that are underutilized [3].

Very long periods of regrowth induce the senescence and death of the first expanded leaf (Table 3). This renders the use of this forage inefficient, resulting in decreased yields, greater senescence losses, and reduced grazing efficiency of the animals, because they will primarily select green leaves.

4.3. Chemical Characteristics

The high values of DM content following treatment with lower N doses (Table 4) were associated with plant age. The forage experienced a longer regrowth period until the time of cutting following treatment with the lower doses of N, which contributed to the increase in DM content. The decreased mineral content due to the increased dose applied is controversial in the literature. Based on the results of the present study, we have no basis to discuss possible reasons for this decrease.

The increase in OM content was due to a decrease in MM. The increase in CP content confirms the ability of grasses such as Guinea grass to respond well to N fertilization, which is due to the high number of leaves in the botanical composition of the harvested fractions that can retain high levels of N in their structure [5]. For the highest dose (50 kg N ha^{-1} application^{-1}), which in this study would be equivalent to an annual dose of 500 kg N ha^{-1} year^{-1}, the CP levels were lower than those levels reported in the literature. Pariz et al. [35], using 50, 100, and 200 kg N ha^{-1} year^{-1}, found crude protein values ranging from 100 to 190 g kg^{-1} DM in four consecutive cuts, which are values higher than those of our study, using N doses lower than ours. Paciolo et al. [34] and Delevatti et al. [36] found maximum doses of nitrogen crude protein levels of 171 g kg^{-1} DM (dose 300 kg N ha^{-1} year^{-1}) and 167 g kg^{-1} DM (dose 270 kg N ha^{-1} year^{-1}), respectively, values higher than ours. This is because in studies that evaluated N doses, the respective doses were divided into an average of four or five applications [34–36]; therefore, the dose per application was much higher than that in the present study, which causes higher levels of CP.

Despite the increase in NDF and HEM contents with increasing N doses, the maximum amplitude of this variation was 2% for NDF and 1% for HEM; we considered that this was insufficient to promote changes in the nutritional aspect and in the consumption of animals that consume these forages. Insua et al. [37] state that senescence is the main factor involved in the changes in NDF; this process of leaf aging was not significant in this experiment. The rainy season reduced the levels of DM and MM, whereas the OM levels increased (Table 4), due to the higher water availability for the forage plant.

The higher CP values (Table 4), as observed in the rainy season, are associated with better edaphoclimatic conditions and high production of leaf blades by the forage plant, because wetter soils favor the use of N [38].

5. Conclusions

In humid tropical climates, the use of N fertilization does not alter the structure of Guinea grass. However, it improves the morphogenic and chemical characteristics, resulting in higher forage accumulation in a shorter period. Furthermore, in the humid tropics, due to the greater regularity of rainfall, there is less variation in the morphogenic, qualitative, and productive parameters of the forage.

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