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Morphogenesis, Structure, and Tillering Dynamics of Tanzania Grass under Nitrogen Fertilization in the Amazon Region

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Abstract: Nitrogen fertilization is one of the main management strategies for continuous pasture management with high productivity. We examined the effects of nitrogen fertilization on the morphogenic, structural, and tillering dynamic characteristics of Tanzania grass in the Amazon region in the state of Pará, Brazil. The study was conducted using a randomized block design with six treatments (0, 100, 200, 300, 400, and 500 kg N ha^{-1} $year^{-1}$) and five replicates. The treatments were performed during the rainy season in 2016 and 2017 using urea as the nitrogen source. The leaf elongation rate was increased by 68.5% compared with that of the control treatment ($p < 0.01$). The leaf appearance rate and number of alive leaves increased with higher doses of nitrogen ($p < 0.01$, each). The regrowth period was reduced by approximately 13 days under 500 kg N ha^{-1} ($p < 0.01$), thus providing more production cycles. Nitrogen fertilization was also associated with a higher tillering rate ($p < 0.01$); however, the rate of this increase decreased with increasing nitrogen dose. Higher nitrogen doses thus improved the development of Tanzania grass; however, this became less pronounced at doses < 300 kg N ha^{-1}.

Keywords: nitrogen; *Panicum maximum*; pasture longevity; tiller; tropical grassland

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1. Introduction

The Amazon is considered the largest agricultural frontier in the world [1], and approximately 25% of cattle herds in Brazil are raised on pastures in this region [2]; however, some production systems still rely on limited management of the soil–plant–animal system. A prominent characteristic of such management is pasture degradation, resulting in reduced productivity, lower stocking rates, and topsoil loss [3]. These processes are typically followed by the conversion to different land use practices and the need for converting previously undisturbed systems to pasture, resulting in greater pressure on the biome [4].

Intensifying ruminant production is one of the alternatives for increasing the competitiveness of this production system compared to other activities, in addition to reducing pressure regarding the deforestation of new areas [5]. Intensification and maintenance of pasture areas can be achieved by improving soil fertility [6], thus enhancing forage production [7,8], improving management, and increasing gains per area [9]. Nitrogen is the main nutrient required for increased pasture productivity, especially in tropical grasses [7,8]. Increased productivity in response to nitrogen availability is due to the biosynthesis of proteins and chlorophyll in plants [10]. Furthermore, nitrogen in the system can help oxidize methane in the soil, thus reducing gas emissions into the atmosphere and reducing adverse effects on the environment [11,12].

The growth-stimulating effects of nitrogen fertilization of pastures are dose-dependent; however, nitrogen use efficiency decreases with increasing nitrogen dose [8]. High nitrogen doses tend to dissipate through ammonia volatilization [13], suggesting that grass growth may decrease with increasing nitrogen fertilization. A recent review paper specified the need to identify the correct dose per application to avoid nitrogen loss to the atmosphere [14].

Several studies on tropical grasses fertilized with nitrogen in Brazil have shown that the maximum dose applied did not produce the best results in the grasses *Megathyrsus maximus* cv. Aruana (with up to 225 kg N ha^{-1}) [15], *Urochloa brizantha* cv. Marandu (up to 450 kg N ha^{-1}) [16], and *Megathyrsus maximus* × *Megathyrsus infestum* cv. Massai (up to 500 kg N ha^{-1}) [8]. We thus hypothesized that the developmental rate of pasture grasses fertilized with nitrogen should decrease at higher doses. The objective of this study was to evaluate the effects of different doses of nitrogen on the grass *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Tanzania with regard to its morphogenic, structural, and tillering characteristics.

2. Materials and Methods

2.1. Location and Planting

This study was conducted in the municipality of Igarapé-Açu, Pará, Brazil, located at the geographic coordinates 01°07′21″ S, 47°36′27″ W, at an altitude of 50 m. Before the experiment, the area was covered with grasses and shrubby vegetation native to the Amazon. The topography of the area was flat and homogeneous. The municipality of Igarapé-Açu experiences an average annual temperature of 26.8 °C and annual precipitation of 3000 mm (Figure 1); the climate is classified as type Am, according to the Köppen Classification [17], characterized as a rainy climate with a short dry period.

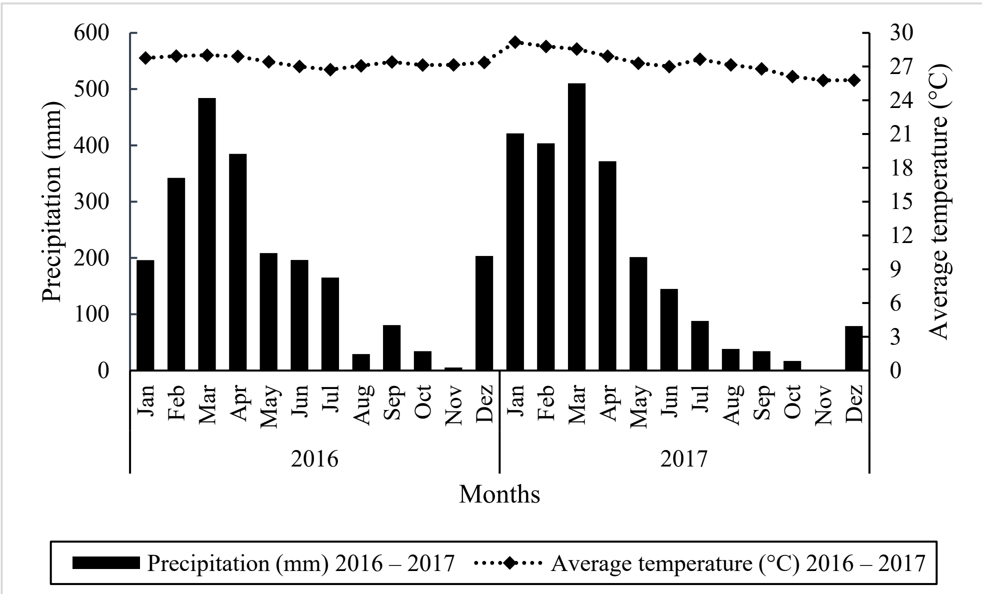

Figure 1. Monthly total rainfall and average temperatures during the study period.

The soil in the experimental area was classified as oxysol with the following chemical composition at a 0–20 cm depth: pH in H_2O: 4.7; organic matter: 7.98 g kg^{-1}; P (Mehlich-1): 1.5 g dm^{-3}; K: 0.03 cmol c dm^{-3}; Ca^{2+}: 0.28 cmol c dm^{-3}; Mg^{2+}: 0.28 cmol c dm^{-3}; and Al^{3+}: 1.11 cmol c dm^{-3}. The soil was amended with dolomitic limestone (TNRP 95%) at a dose equivalent to 2.9 tons ha^{-1} to increase the base saturation to 60% and allow cultivation of Tanzania grass.

After preparing the soil, Tanzania grass seeds were planted under phosphate fertilization (simple superphosphate) at a dose equivalent to 80 kg of P_2O_5 ha^{-1}, applied by haul. Periodic cuts were performed to a residual height of 35 cm for adequate pasture management. The experiments began in January 2016, and phosphate fertilizer was applied again with potassium fertilizer at a dose equivalent to 60 kg K_2O ha^{-1} (in the form of KCl). The first application of nitrogen was applied in each treatment.

2.2. Treatments and Experimental Design

A randomized block design was used with six treatments corresponding to nitrogen doses of 0 (control), 100, 200, 300, 400, and 500 kg N ha^{-1} year^{-1}. Five replicates per treatment were used, resulting in 30 experimental units. The plots were 12 m^2 (3 m $\times$ 4 m), separated by 1 m corridors. Measurements were conducted over two consecutive years (2016 and 2017) during the rainy period (January–July).

Nitrogen and potassium were applied six times at intervals of 30 days. Fertilizer application began in January and continued until June.

2.3. Management of Experimental Plots

The height of the Tanzania grass canopy was measured in centimeters using a ruler, and the average canopy height was calculated using 10 points per plot. At a grass height of 70 cm, each plot was cut to a height of 35 cm; at 70 cm height, the canopy reached 95% light interception [18,19] and a balance between productivity and quality occurs in Tanzanian grass. On 8 January 2016, a leveling cut to a residual height of 35 cm was performed in all plots, and the nitrogen treatments were applied.

2.4. Experimental Measurements

After the leveling cut, five tillers in the vegetative phase (as per the average conditions of the plot) were marked in each plot. The tillers were identified using plastic ties, numbered, and measured using a graduated ruler. Tiller monitoring was performed twice per week (on Wednesdays and Saturdays). New tillers were marked, and the plots were cut according to the same initial criteria.

The leaves were numbered and classified as fully expanded (fully expanded and with a visible ligule), expanding (no visible ligule), or senescent (when the leaf blade showed signs of senescence). The leaves were considered dead when 50% of the leaf blade showed signs of senescence. The stem length was measured from the ground to the ligules of the last fully expanded leaf [20]. A graduated ruler was used for all measurements.

The following morphogenic and structural variables were examined: (i) leaf elongation rate (LER; cm of leaf tiller^{-1} day^{-1}); (ii) leaf appearance rate (LAR; leaves tiller^{-1} day^{-1}); (iii) phyllochron (PHY; days leaf^{-1} tiller^{-1}); (iv) leaf senescence rate (LSR; cm of leaf tiller^{-1} day^{-1}); (v) leaf life span (LLS; days); (vi) stem elongation rate (SER; cm of stem tiller^{-1} day^{-1}); (vii) final leaf size (FLS; cm); (viii) number of living leaves (NLL; leaves tiller^{-1}).

The tiller population density (TPD; tiller m^{-2}) was evaluated by double-sampling the total number of tillers (basal and aerial) existing in 0.5 m $\times$ 1.0 m frames, positioned at points that represented the average condition of the plot. Counts were performed every 30 days.

A PVC ring measuring 25 cm in diameter was inserted inside a clump in each plot to evaluate the tiller patterns. In the first assessment (generation zero; G0), all tillers inserted inside the ring were marked with single-color wires to identify the generation. Dead tillers were counted every 30 days, and the tillers that were produced in subsequent generations (G1–G6) were marked using wires of different colors.

Based on these assessments, we calculated the following: (i) tiller appearance rate (TAR; tiller 100 tiller^{-1} day^{-1}) = (number of new tillers/total number of tillers from the previous generation)/30; (ii) tiller mortality rate (TMR; tiller 100 tiller^{-1} day^{-1}) = (number of new tillers/total number of tillers from the previous generation)/30; and (iii) tiller survival rate (TSR; tiller 100 tiller^{-1} day^{-1}) = 1 − TMR. The pasture stability index (P1/P0) was calculated as described previously [21], using the equation P1/P0 = TSR × (1 + TAR).

2.5. Statistical Analyses

The data were analyzed using Shapiro–Wilk and Levene's tests to confirm normality of residuals and homogeneity of variances, respectively. An analysis of variance was performed ($p < 0.05$), and orthogonal polynomial contrasts were used to determine the nature of the responses according to the application of nitrogen. All statistical analyses were performed using R statistical software (version 4.0.2; R Core Team, 2014).

3. Results

The morphogenic and structural variables LER, LAR, PHY, SER, and NLL showed significant changes ($p < 0.05$, each) as the nitrogen dose increased (Table 1). The LSR, LLS, and FLS variables were not significantly different.

Table 1. Morphogenic and structural characteristics of Tanzania grass at various doses of nitrogen fertilization.

Variable	Nitrogen Doses (kg N ha^{-1})						SEM	*p*-Value	Contrast	
	0	100	200	300	400	500			L	Q
LER	2.505	2.706	3.416	3.207	3.680	4.220	0.627	<0.01	<0.01	0.771
LAR	0.066	0.073	0.079	0.077	0.086	0.083	0.015	0.031	0.049	0.751
PHY	15.495	14.782	13.484	12.304	11.169	10.372	1.844	<0.001	<0.001	0.990
LSR	0.419	0.462	0.401	0.463	0.469	0.497	0.105	0.818	0.228	0.711
LLS	41.023	44.091	43.665	40.047	36.628	37.040	7.886	0.618	0.133	0.443
SER	0.072	0.072	0.081	0.082	0.112	0.109	0.026	0.035	<0.001	0.532
FLS	21.381	23.787	22.590	21.874	22.884	22.640	1.761	0.533	0.673	0.547
NLL	2.825	3.599	3.433	3.389	3.475	3.873	0.253	<0.001	<0.001	0.397
RP	33.2	27.5	24.04	24.64	21.56	19.52	2.362	<0.001	<0.001	0.060
NC	6.2	7.4	8.8	8.6	9.4	10.6	0.809	<0.001	<0.001	0.477

LER: leaf elongation rate; cm leaf tiller^{-1} day^{-1}; LAR: leaf appearance rate; leaf tiller^{-1} day^{-1}; PHY: phyllochron; days leaf^{-1} tiller^{-1}; LSR: leaf senescence rate; cm leaf tiller^{-1} day^{-1}; LLS: leaf life span; days; SER: stem elongation rate; cm stem tiller^{-1} day^{-1}; FLS: final leaf size; cm; NLL: number of living leaves; leaf tiller^{-1}; RP: regrowth period; days; NC: number of cycles; SEM: standard error of the mean; L: linear; Q: quadratic.

An increasing linear response was observed in LER ($p < 0.01$), with values of 2.505 and 4.220 cm leaf tiller $^{-1}$ day^{-1} for doses of 0 and 500 kg ha^{-1}, respectively. An increase of 0.0084 cm in leaf tiller^{-1} day^{-1} was observed for each kilogram of nitrogen applied. LAR showed a linear increase ($p = 0.049$). LAR increased linearly ($p < 0.05$) with increasing nitrogen dose, at a difference of approximately 25% for the highest doses. PHY showed an opposite trend to the other variables, with a decrease ($p < 0.01$) depending on the nitrogen dose.

SER increased ($p < 0.01$) with increasing nitrogen dose, at a difference of approximately 155% for the highest doses. The NLL showed a linear effect ($p < 0.05$) with increasing nitrogen doses.

The canopy regrowth period decreased ($p < 0.01$) as the nitrogen dose increased. Without fertilization, regrowth lasted approximately 33 days, which was approximately 14 days shorter than that at the highest dose (500 kg ha^{-1}). The number of cycles increased ($p < 0.01$) according to the nitrogen dose, with up to 4.4 cycles more at the highest nitrogen dose.

As for the variables related to tillering (Table 2), TDP showed a quadratic behavior ($p < 0.05$), with an accelerated rate of tillering up to a dose of 300 kg ha^{-1}, which decreased at 400 and 500 kg N ha^{-1}. An increase of approximately 14% in tillering was observed by applying nitrogen fertilization (100 kg N ha^{-1}); however, as the dose increased, the difference compared to the previous application tended to decrease, reaching an approximately 1% difference between the dose of 400 and 500 kg of N ha $^{-1}$. TAR, TMR, and

P1/P0 showed a linear response ($p < 0.05$) to an increase in nitrogen dose. The TSR was not affected by nitrogen application ($p > 0.05$).

Table 2. Tiller population dynamics of Tanzania grass at various doses of nitrogen fertilization.

Variable	Nitrogen Doses (kg N ha^{-1})						SEM	p-Value	Contrast	
	0	100	200	300	400	500			L	Q
TPD	284.0	432.7	518.9	523.6	642.7	692.1	36.06	<0.01	<0.01	0.059
TAR	0.115	0.135	0.137	0.140	0.137	0.150	0.001	0.041	0.012	0.438
TMR	0.127	0.104	0.108	0.102	0.095	0.098	0.002	0.020	0.029	0.350
TSR	0.988	0.990	0.989	0.989	0.991	0.990	0.002	0.259	0.064	0.701
P1/P0	0.999	1.003	1.003	1.003	1.005	1.005	0.002	0.003	<0.01	0.304

TPD, tiller population density; tiller m^{-2}; TAR, tiller appearance rate; tiller 100 tiller^{-1} day^{-1}; TMR, tiller mortality rate; tiller 100 tiller^{-1} day^{-1}; TSR, tiller survival rate; tiller 100 tiller^{-1} day^{-1}; P1/P0, pasture stability index; SEM, standard error of the mean; L, linear; Q, quadratic.

Increased tillering was observed during the initial months at all fertilization levels (Figure 2). A balance occurred between the appearance and mortality of offspring; tillers at low levels of nitrogen fertilization showed less pronounced responses than those in other treatments. The variation in tillering dynamics when nitrogen fertilization was not applied was smaller than that in the other treatments receiving fertilization.

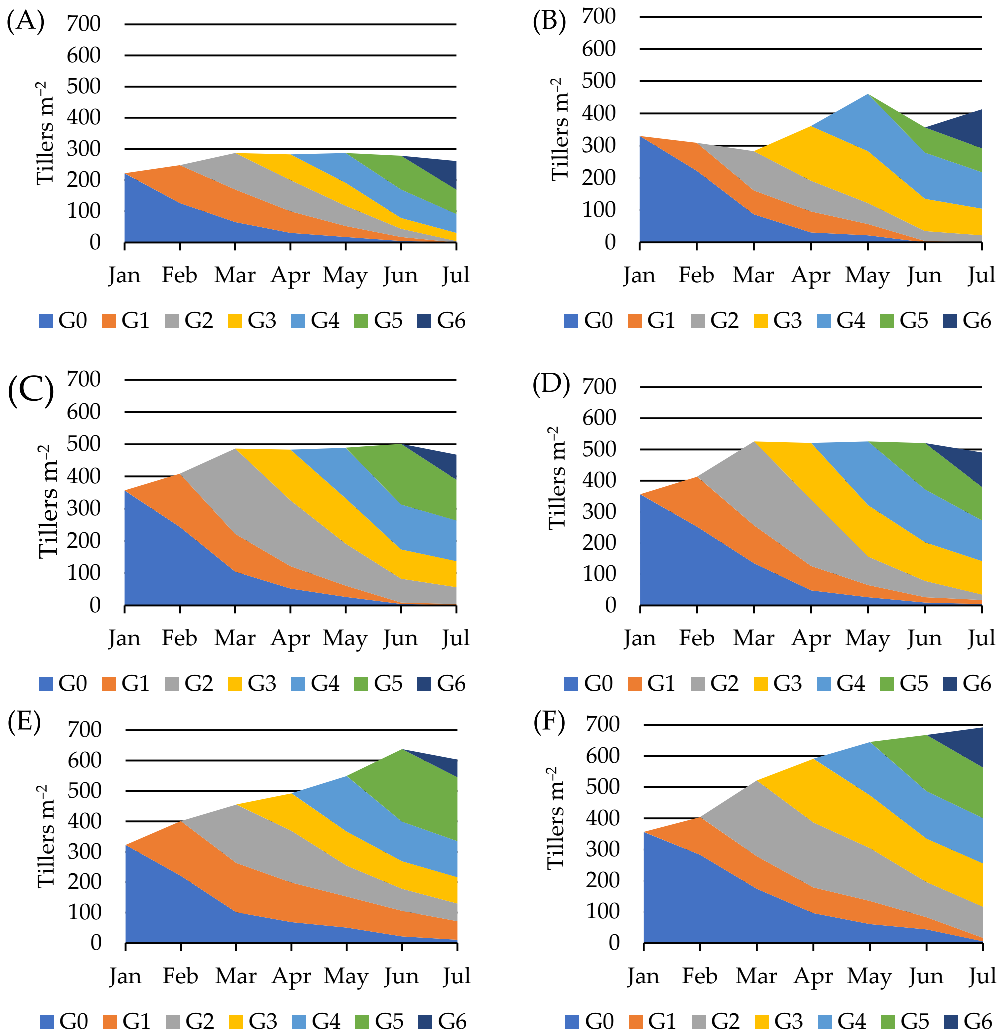

Figure 2. Demographic pattern of Tanzania grass tillers subjected to nitrogen doses 0 (**A**), 100 (**B**), 200 (**C**), 300 (**D**), 400 (**E**), 500 (**F**) kg of N ha^{-1}. G: generation of tillers.

4. Discussion

Temperature, light, water availability, and soil nutrients are key elements for optimal photosynthetic efficiency in forage grasses. In the northern Amazon region, constant temperatures throughout the year, high luminosity, and well-distributed rainfall [11] result in good photosynthetic rates; however, the supply of nutrients is suboptimal, thus negatively influencing productivity. In this context, nitrogen, a constituent element of proteins, chlorophyll, and essential biochemical compounds for the growth of forage plants, is crucial for the morphogenesis, structure, and tillering of grasses, accelerating tissue flow, and stimulating basal buds.

In the current study, the highest LER occurred as a result of nitrogen accelerating the pace of cell division and cell expansion, leading to greater production of photosynthate, which is responsible for the translocation of sucrose in the plant, providing more energy for the grass to lengthen its leaves [22–25]. These results corroborate those of previous studies on the grass species *Megathyrsus maximus*, cultivars Tamani, Mombaça, and Massai [7,8,26]. These studies showed average values of up to 3700 cm for leaf tiller^{-1} day^{-1}; however, the increase in LAR was due to the same reasons, as the apical meristem has a high demand for nitrogenous compounds, which provide greater leaf release [27] and, consequently, accelerated leaf appearance, thus reducing the phyllochron of the forage plant [8].

Despite the accelerated rate of plant growth, the LSR was not influenced by nitrogen fertilization, which was due to the plant being cut at the ideal height (70 cm), after which the net photosynthesis decreased owing to the greater shading by older leaves [28]. Leaf senescence is considered the last stage of a plant's useful life; therefore, the lack of difference in LSR explains why LLS did not provide a response, as the plants distributed photoassimilates uniformly among all leaves, maintaining the lifespan of the plant [29].

Upon reaching the ideal grazing point (95% light interception or approximately 70 cm in height), competition for light between tillers increases in the forage canopy [30]. This results in a greater SER because tillers tend to seek light by lengthening their stems [8,30,31]. Further, the culm is the plant's support structure; therefore, the greater the appearance and elongation of the leaves, the more the culm needs to resist these changes, as observed for the variables LER and LAR [15].

NLL depends directly on the environmental conditions to be appropriately expressed [32]. Such responses can be observed in LAR, which, under higher fertilization, showed a greater appearance of leaves in addition to SER, showing that a larger number of leaves causes an elongation of the stem because of the shading of lower leaves.

After cutting, the fertilization treatments had greater nutritional reserves to reconstitute the leaf area, thus accelerating regrowth. Therefore, higher fertilization levels corresponded to shorter times for the plant to reach the optimal cutting height [33]. Owing to the faster regrowth rate, a greater number of harvest cycles could also be observed, similar to results reported previously [7,8,34].

Tillering is an important mechanism for maintaining the perenniality of a pasture, adjusting the leaf area index, and maintaining the photosynthetic efficiency of forage [35]. Our results corroborate those observed in a previous study on Mulato grass in rotational stocking [36], showing that regardless of the residue height, nitrogen fertilization results in greater tillering. Rapid tillering at the beginning of January (Figure 2) resulted from offspring renewal [14]. During the dry period, tillers tend to remain longer as a form of survival; therefore, the existence of older tillers is natural. Upon entering the rainy season, the renewal of tillers as a result of the entry of water into the soil, together with the application of nitrogen, maximizes tillering [36]. Moreover, grasses fertilized with higher doses of nitrogen facilitate a higher number of regrowth cycles, which provides greater light entry at the base of the canopy. Such light entry stimulates tiller buds, allowing for greater tillering [37].

The effects observed on TAP show the influence of nitrogen on the dormant buds of grass, thus increasing tillering [35,38]. Many plants, including forage grasses, exhibit phenotypic plasticity, which is the ability to change phenological characteristics to adapt to environmental stimuli, including damage [39,40]. In the present study, tillering may be considered a strategy to store reserves for tillering after cutting. The higher the dose of nitrogen applied, the higher the nutrient levels in the soil and cutting frequencies; that is, the plants suffered damage and had nutritional reserves to adapt to such stress.

The pasture stability index is based on the total population of tillers over time, and values < 1.0 indicate that the appearance of tillers does not compensate for the death of tillers and may cause an imbalance in the pasture in the future. The highest TAR and lowest TMR in the treatments that received higher levels of fertilizer showed this positive response. The results showed that nitrogen fertilization can maintain the perenniality of the pasture for a longer period, owing to the greater appearance of tillers and their survival, as discussed previously [38].

5. Conclusions

Increasing nitrogen doses accelerated the metabolism of Tanzania grass, providing a greater increase in leaf tissue and reducing the plant's regrowth time, which positively affected the leaf elongation, leaf appearance, and tiller mortality rates; however, the plant response decreased as the dose increased, and nitrogen use efficiency tended to decrease above doses of 300 kg ha^{-1} year^{-1}. It is important to highlight that, depending on the environmental condition, different answers can be found, therefore it is necessary to study the area where production will begin.

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