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Research Paper

Effects of timing of nitrogen fertilizer application on responses by tropical grasses

Efectos del tiempo adecuado de fertilización con nitrógeno en la respuesta de gramíneas tropicales

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Abstract

Timing of nitrogen (N) fertilizer application can influence grass regrowth, so it is important to identify how tropical grasses respond to delays in applying fertilizer after defoliation. Our objective was to identify the effects of timing of N fertilizer application after harvest on the productive, morphogenic and structural characteristics of 3 tropical grasses: 'Xaraés' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Xaraés), 'Marandu' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Marandu) and 'Tanzânia' (*Megathyrsus maximus* [Jacq.] cv. Tanzânia). The experiments were performed in a greenhouse, in a completely randomized design, with 5 delays in applying N after harvesting (0, 3, 6, 9 and 12 days). Delaying fertilizer application did not affect the forage mass of Xaraés and Marandu palisade grass (7.4 and 7.8 g/pot, respectively). There was a linear decrease in number of leaves per tiller and leaf appearance rate, but tiller population density and phyllochron increased linearly as fertilizer application was delayed. Grass forage mass (12.2–10.6 g/pot), number of leaves per tiller (3.1–2.6 leaves/tiller) and forage accumulation rate (0.47 to 0.41 g DM/d) of Tanzânia guinea decreased linearly as N application was delayed, but tiller population density was unaffected (25 tillers/pot). Based on our results, N fertilizer should be applied to Tanzânia guinea grass pastures as soon as possible after harvest and certainly before 3 days, while there is not the same urgency with Xaraés and Marandu where fertilization could be delayed up to 12 days without significant detriment. These suggestions need to be tested in a field study before being recommended widely.

Keywords: Maintenance fertilizer, *Megathyrsus maximus*, nitrogen fertilizer, root mass, tiller density, tropical pastures, *Urochloa brizantha*.

Resumen

El momento de la fertilización nitrogenada puede influir en la regeneración de la pastura, lo que requiere identificar cómo responden las gramíneas tropicales a la fertilización después de una defoliación. Por lo tanto, nuestro objetivo fue identificar el impacto del tiempo adecuado de fertilización nitrogenada en las características productivas, morfogénicas y estructurales después de cosechar tres pasturas tropicales: 'Xaraés' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Xaraés), 'Marandu' (*Urochloa brizantha* [Hochst. ex A. Rich.] Stapf cv. Marandu) y 'Tanzânia' (*Megathyrsus maximus* [Jacq.] cv. Tanzânia).

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Los experimentos se realizaron en invernadero, en un diseño completamente al azar, con cinco tiempos de fertilización nitrogenada (0, 3, 6, 9 y 12 días). El tiempo de fertilización nitrogenada no afectó la masa forrajera de los pastos Xaraés y Marandu (7.4 y 7.8 g/maceta, respectivamente). Hubo una disminución lineal en el número de hojas por macolla y la tasa de aparición de la hoja, sin embargo, la densidad de población de macolla y el filocrón aumentaron linealmente de acuerdo con el momento de la fertilización nitrogenada. La masa de forraje de Tanzânia guinea (12.2 a 10.6 g/maceta), el número de hojas por macolla (3.1 a 2.6 hojas/macolla) y la tasa de acumulación de forraje (0.47 a 0.41 g/d) disminuyen linealmente de acuerdo con el tiempo de fertilización nitrogenada, sin embargo, la densidad poblacional de la macolla fue similar (25 rebrotes/maceta). Según nuestros resultados, el fertilizante N debe aplicarse a los pastizales de pasto Tanzânia guinea tan pronto como sea posible después de la cosecha y ciertamente antes de los 3 días, mientras que la fertilización de los cultivares de *B. brizantha* se puede realizar hasta 12 días después de la cosecha. Estas sugerencias deben probarse en un estudio de campo antes de ser recomendadas ampliamente.

Palabras clave: Densidad de rebrotes, fertilización de mantenimiento, fertilización nitrogenada, masa radicular, *Megathyrsus maximus*, *Urochloa brizantha*.

Introduction

Grasslands are among the most abundant ecosystems in the world (Hewins et al. 2018), where livestock production occurs in areas with native or exotic grasses. As a result, pastures are the main forage source in animal production systems in most tropical countries (Jank et al. 2014), with wide diversity in grazing systems, species and grazing management.

Periodic soil nutrient replacement is important, especially in intensified systems with high stocking rate, (Bourscheidt et al. 2019) which is commonly a combination of intermittent stocking management and a grass with high potential production. Thus, fertilizer application becomes an essential practice, especially when pastures have been established in infertile soils, such as the Oxisols (Chaves et al. 2017).

In grasslands, nitrogen (N) is the most limiting nutrient for plant growth, because it is one of the most extracted nutrients by grasses (Costa et al. 2016) and is a constituent of many cell components associated with the photosynthetic process (Taiz et al. 2017). The N requirement of grass is greater than that of other nutrients, so applying N fertilizer should be given priority to maintain pasture productivity and persistence (Rosado et al. 2017).

Timing of application of N fertilizer can be an important factor affecting grass regrowth (Marques et al. 2016), especially in highly productive systems. Immediately after harvesting, there is a period during which the grass is less responsive to N fertilizer due to the reduction in leaf area and root mass, where tillers need to rely on organic reserves (Faria et al. 2019). Each cultivar has a genetic characteristic driving the recovery potential after harvest and the timing of fertilizer application can contribute to enhancing regrowth (Gomide et al. 2019) and productivity, especially in systems where plants have high demand for N.

The most efficient usage of N has been reported in pastures under intermittent stocking, where adequate N results in rapid regrowth. Faster regrowth after N fertilizer application is attributed to the increased leaf appearance rate (LAR) and, therefore, reduction in phyllochron (Soares Filho et al. 2015; Paciullo et al. 2017). Although there are many studies in the literature on the optimal rates of N to apply to pastures, there is limited information regarding optimal timing of N application.

Approximately 85% of Brazil's pasture lands are cultivated with Urochloa (formerly: *Brachiaria*) species (Valle et al. 2014). The second most important genus is *Megathyrsus*, which is recommended for systems with medium to high fertility soils (Muir and Jank 2004). 'Xaraés' palisade grass (*Urochloa brizantha* syn *Brachiaria brizantha* cv. Xaraés), 'Marandu' (*Urochloa brizantha* syn *Brachiaria brizantha* cv. Marandu) and 'Tanzânia' guinea grass (*Megathyrsus maximus* syn *Panicum maximum* cv. Tanzânia) are widely used in forage-livestock systems in Central Brazil (Jank et al. 2011; 2014).

With the overall goal of increasing efficiency of N fertilizer usage in forage-livestock systems, we hypothesized that timing of applying N fertilizer can affect regrowth and, consequently, productivity of pastures. Therefore, our objective was to identify the effects of different timing of N fertilizer application after harvesting on the productive, morphogenic and structural characteristics of 3 tropical grasses (Xaraés, Marandu and Tanzânia).

Materials and Methods

Three experiments were carried out in a greenhouse at Federal University of Mato Grosso, Cuiabá, Mato Grosso State, in a completely randomized design with 5 treatments and 12 replicates. The experiments were conducted from April to December 2016, where each experiment studied the effects in one grass: Experiment I –Xaraés palisade grass; Experiment II – Marandu palisade grass; and Experiment III – Tanzânia guinea grass. In all experiments, treatments were 5 times post-harvest for applying N fertilizer (0, 3, 6, 9 and 12 days), where zero represented application immediately after harvest, i.e. on the same day. The same procedure was followed for each experiment.

The soil was an Oxisol (Santos et al. 2018) and was collected from the 0–20 cm layer and chemical and granulometric analyses were performed according to Teixeira et al. (2017): pH (H_2O): 6.5; P: 7.9 mg/dm³; K: 25.3 mg/dm³; Ca: 1.7 cmolc/dm³; Mg: 0.6 cmolc/dm³; H+Al: 1.2 cmolc/dm³; CEC: 3.5 cmolc/dm³; base saturation: 66%; organic matter: 9.7 mg/dm³; sand: 790 g/kg; silt: 41 g/kg; and clay: 169 g/kg.

The soil was sieved in a 4 mm mesh and used to fill plastic pots (5 dm^3 experimental units). Phosphorus was applied at sowing at the rate of 87 mg P/dm^3 in the form of simple superphosphate. Twenty seeds of each grass were planted in each pot, and 10 days after sowing, the plants were thinned to leave 5 plants per pot, using plant uniformity as the criterion. Fifteen days after thinning, 30 mg K_2O/dm³ (25 mg K/dm^3) in form of potassium chloride and 50 mg N/dm³ in form of urea was applied to all treatments.

Thirty-five days after thinning, plants in all pots were clipped at 15-cm stubble height for Marandu and Xaraés and 30 cm for Tanzânia. This was considered day 0 for each experiment, when 100 mg N/dm^3 (as urea) was applied to the Control and, following the chronogram, the same N fertilizer regime (dose and source) was applied at 3, 6, 9 and 12 days after clipping for the various treatments.

Four regrowth cycles (27-d) were evaluated for Xaraés palisade grass (April 22, May 18, June 14 and July 11), and 3 regrowth cycles for Marandu palisade grass (June 13, July 10 and August 6) and Tanzânia guinea grass (September 18, October 15 and November 11). For the third regrowth cycle of all experiments, 25 mg K/dm^3 was applied to reduce the incidence of foliage necrosis at leaf edges attributed to K deficiency.

The gravimetric method was used for irrigation, maintaining soil moisture near field capacity, as described by Cabral et al (2018).

Measurements

Every 27 days (last day of the regrowth cycle), Xaraés and Marandu were clipped at 15 cm and Tanzânia at 30 cm. Prior to clipping, in each pot, canopy height was measured with a graduated rule, and number of tillers and number of leaves (exposed ligule) above the clipping height were counted. The number of leaves per tiller (NLT) was obtained by dividing number of leaves (NL) by number of tillers in each pot. Leaf appearance rate (LAR) was estimated by the ratio between NLT and the interval between harvests. Phyllochron (PHY) was the inverse of LAR.

Forage mass (FM) was separated into morphological components, i.e. leaf (LM), stem (sheath + pseudostem; SM) and dead material (Dead). No dead material was observed in Marandu and Tanzânia in any regrowth cycle. Morphological components were dried at 55 °C in a forced-air dryer for 72 hours and weighed. Forage accumulation rate (FAR) was calculated by dividing FM by the length of the rest period. The mean weight of a leaf (individual leaf mass; ILM) was estimated by dividing leaf dry mass by NL. Individual tiller mass (ITM) was obtained by dividing FM by the number of tillers.

In the last regrowth period for each experiment (the third for Marandu and Tanzânia and the fourth for Xaraés), after harvesting the forage mass, the residue and root masses were collected. The soil was washed away on 4-mm sieves and the roots were dried following the same drying procedure and root mass (RM) was estimated.

Statistical analyses

Data were analyzed using general linear mixed model method, using the PROC MIXED command (SAS® Institute Inc., Cary, NC). Timing of N fertilizer application was considered a fixed effect. Regrowth cycle and replication (blocks) were considered random effects. Degrees of freedom were corrected using the Satterthwaite method and the variance and covariance structures were chosen based on Akaike Information Criterion. An orthogonal polynomial contrast ($P \leq 0.05$) was used to evaluate the effects (linear or quadratic) of timing of N fertilizer application.

The model used was as follows:

$y_{ijk} = \mu + T_i + e_{ij} + C_k + \varepsilon_{ijk}$;

where:

y_{ijk} = expected response;

μ = average/constant, associated with the experiment;

T_i = treatment effect (fertilizer timing) i;

e_{ij} = treatment error i, in replicate j, normally and independently distributed;

C_k = random effect associated with regrowth cycle k, normally distributed; and

ε_{ijk} = experimental error associated with treatment i, in replicate j, in cycle k, normally distributed.

When significant for the quadratic effect, the non-linear regression procedure (PROC NLIN) of the SAS® software was used to identify whether a plateau ($P \leq 0.05$) was reached for timing of N fertilizer application.

Principal component analysis (PCA) was performed and clusters were constructed using R software, v 4.0.2 (R Development Core Team 2015) and 'Cluster', 'FactoMiner' and 'Factoextra' packages, in order to characterize the response variables for the grasses and the N fertilizer timing. The biplots resulting from PCA are interpreted by visualizing inversely correlated quadrants (vectors in opposite directions). The larger the size of the arrows (vectors), the greater the variation in the data, and the closer the vectors, the greater the relationship between them. With the same database used for PCA analysis, one cluster analysis was run for Xaraés (X), Marandu (M) and Tanzânia (T), at different timing of N application (0, 3, 6, 9 and 12 days) being shown in the factor map. A heatmap ('heatmap.2' command, 'gplots' package) was constructed, and to facilitate the reading of the heatmap, cluster analyses were performed using Euclidean distance.

Results

Marandu and Xaraés palisade grass

Timing of N fertilizer application did not affect (P>0.05) FM, LM, SM, plant height and NL for Xaraés and Marandu (Tables 1 and 2).

There was a linear effect of N application timing on Dead, LAR and PHY for Xaraés (Table 1) and Marandu (Table 2). With increase in time elapsed following harvesting before N was applied, PHY increased but LAR decreased.

Individual tiller mass and TPD were affected by the length of delay in applying N for both Xaraés and Marandu (Tables 1 and 2). While ITM in Xaraés showed a quadratic effect, with a lesser value for the 9 days delay in applying N after harvest, linear effects were observed with TPD for Xaraés and Marandu and NLT and ITM for Marandu.

Tanzânia guinea grass

Delaying N application after harvest did not affect (P>0.05) SM, ILM, RM and LAR for Tanzânia (Table 3). However, delaying N application had linear effects on FM, LM, NL, FAR and PHY with the relationship being positive for PHY but negative for FM, LM, NL and FAR (Table 3). Plant height and length of delay followed a quadratic relationship, with the lowest point reached between 6 and 9 days delay after harvest (Table 3).

ITM and NLT for Tanzânia guinea grass were negatively and linearly related to length of delay in applying N after harvest (Table 3) but TPD was not affected (mean 25.2 tillers/pot).

Common responses

The only variables affected similarly by timing of N fertilizer application after harvest in all 3 grasses were ITM and PHY. The latest fertilizer application (12 days after harvest) caused a reduction in NLT and an enhancement in PHY.

Table 1. Productive, morphogenic and structural variables of Xaraés palisade grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	6.8	7.1	7.9	7.4	7.8	0.07	0.36	-	1.6
LM (g DM/pot)	4.0	4.5	5.0	4.5	4.9	0.10	0.28	-	0.4
SM (g DM/pot)	1.2	1.0	1.1	1.1	1.1	0.61	0.14	-	0.5
Dead (g DM/pot)	1.6	1.7	1.8	1.9	1.9	0.003	0.32	-	1.4
ITM (g DM)	0.32	0.40	0.36	0.29	0.30	0.02	0.01	0.78	0.1
ILM (g DM)	0.08	0.09	0.11	0.08	0.09	0.10	0.06	-	0.01
RM (g DM/pot)	11.8	4.9	9.9	7.9	12.5	0.49	0.05	0.46	2.3
Morphogenic and structural characteristic									
Height (cm)	47	47	48	49	48	0.42	0.57	-	3
NLT	1.6	1.8	1.6	1.6	1.6	0.20	0.25	-	0.2
LAR (leaves/tiller/d)	0.08	0.107	0.078	0.076	0.075	0.002	0.32	-	0.008
NL (leaves/pot)	48	46	50	54	54	0.13	0.62	-	3
TPD (tillers/pot)	30	26	31	33	33	<0.0001	0.15	-	2
FAR (g DM/d)	0.25	0.26	0.29	0.27	0.29	0.07	0.36	-	0.06
PHY (days/leaf)	12.3	10.7	13.3	13.6	14.0	0.001	0.27	-	1.3

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12 = number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear; Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; Dead: dead material mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

Table 2. Productive, morphogenic and structural variables of Marandu palisade grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	7.6	7.8	7.7	7.9	7.8	0.45	0.71	-	0.9
LM (g DM/pot)	5.8	6.0	6.0	5.9	6.2	0.36	0.95	-	0.5
SM (g DM/pot)	1.7	1.8	1.7	1.9	1.7	0.80	0.29	-	0.4
ITM (g DM)	0.32	0.33	0.28	0.30	0.26	<0.0001	0.33	-	0.04
ILM (g DM)	0.09	0.09	0.09	0.09	0.09	0.75	0.82	-	0.01
RM (g DM/pot)	12.7	12.5	12.8	11.6	17.8	0.15	0.14	-	2.0
Morphogenic and structural characteristic									
Height (cm)	48	48	47	49	49	0.31	0.25	-	4.15
NLT	2.5	2.6	2.2	2.3	2.1	<0.0001	0.74	-	0.1
LAR (leaves/tiller/d)	0.092	0.098	0.081	0.084	0.079	<0.0001	0.83	-	0.005
NL (leaves/pot)	59	62	62	60	65	0.23	0.73	-	3.73
TPD (tillers/pot)	24	24	28	27	31	<0.0001	0.69	-	2.26
FAR (g DM/d)	0.3	0.3	0.3	0.3	0.3	0.45	0.71	-	0.03
PHY (days/leaf)	11.2	10.6	12.8	12.2	12.6	<0.0001	0.57	-	0.8

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12: number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear; Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

Table 3. Productive, morphogenic and structural variables of Tanzânia guinea grass according to time after harvesting before nitrogen fertilizer was applied.

Parameter	Nitrogen fertilizer timing (d)[1]					P-value[2]			s.e.m.
	0	3	6	9	12	L	Q	P	
Productive characteristic									
FM (g DM/pot)	12.2	11.8	11.4	10.4	10.6	<0.0001	0.59	-	1.1
LM (g DM/pot)	12.2	11.8	11.4	10.4	10.5	<0.0001	0.64	-	0.7
SM (g DM/pot)	0.04	0.02	0.06	0.02	0.08	0.42	0.38	-	0.15
ITM (g DM)	0.54	0.49	0.50	0.45	0.46	0.0009	0.37	-	0.54
ILM (g DM)	0.18	0.16	0.18	0.16	0.17	0.12	0.34	-	0.16
RM (g DM/pot)	26.7	35.7	33.2	23.4	26.3	0.44	0.34	-	5.2
Morphogenic and structural characteristic									
Height (cm)	70	69	68	66	72	0.01	0.01	0.93	2
NLT	3.1	3.1	2.8	2.8	2.6	0.002	0.97	-	0.2
LAR (leaves/ tiller/d)	0.11	0.11	0.10	0.10	0.09	0.10	0.74	-	0.01
NL (leaves/pot)	71	76	66	67	64	0.01	0.60	-	4
TPD (tillers/pot)	24	26	25	25	26	0.51	0.95	-	3
FAR (g DM/d)	0.47	0.45	0.44	0.40	0.41	0.0019	0.71	-	0.003
PHY (days/leaf)	9.1	9.1	10.0	10.1	10.7	0.001	0.81	-	0.7

[1]Timing of N fertilizer application after harvesting (0: immediately after harvest; 3, 6, 9 and 12 = number of days after harvest).
[2]Orthogonal polynomial contrast: L = linear, Q = quadratic; Non-linear regression: P = plateau.
FM: forage mass; LM: leaf mass; SM: stem mass; ITM: individual tiller mass; ILM: individual leaf mass; RM: root mass; NLT: number of leaves per tiller; LAR: leaf appearance rate; NL: number of leaves; TPD: tiller population density; FAR: forage accumulation rate; PHY: phyllochron.

The PCA explained 90.6% of the data variance in PC1 and PC2 (Figure 1). PC1 expressed 79.7% of the data's variability, suggesting that this axis was sufficient to explain all variability (Figure 1). In this PCA, LM, FM and FAR were more correlated (0.96, 0.92 and 0.92, respectively).

The factor map indicates a solution of 2 clusters (Figure 2). The combinations identified in Cluster 1 included Tanzânia guinea grass at different N application timings. The combinations that were grouped in Cluster 2 included Marandu and Xaraés palisade grass at different N application timings. Cluster 1 is characterized by greater values for height, LM, ILM, FM, FAR, ITM, LAR, NLT and LN (variables classified among the strongest, Figure 1) and lesser values for the variables SM and PHY (variables classified among the weakest, Figure 1). Cluster 2 is characterized by greater values for SM and PHY (variables classified among the strongest, Figure 1) and lesser values for height, LM, ILM, FM, FAR, ITM, LAR, NLT and LN (variables classified as the weakest, Figure 1).

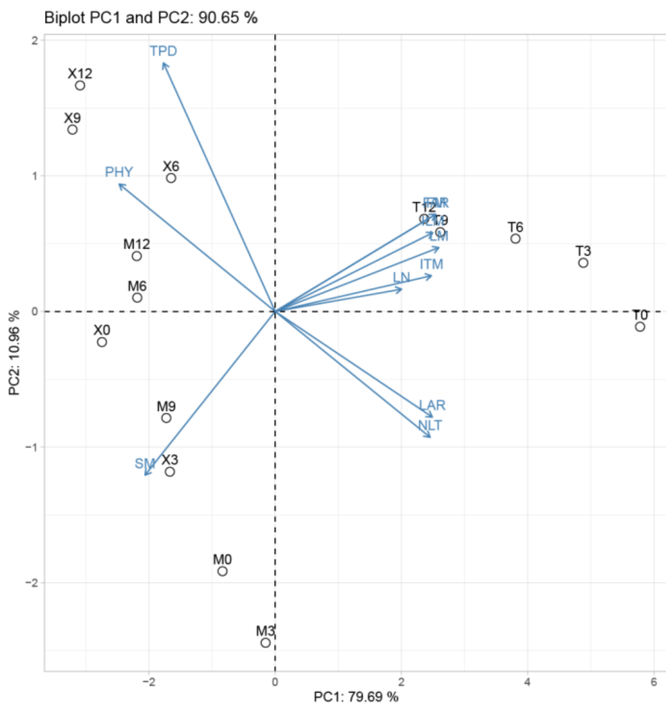

Figure 1. Biplot for the productive and morphogenic characteristics of Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass with different delays in applying nitrogen fertilizer after harvest (0, 3, 6, 9 and 12 days). Height (cm); FM: forage mass (g DM/pot); LM: leaf mass (g DM/pot); SM: stem mass (g DM/pot); ITM: individual tiller mass (g DM); ILM: individual leaf mass (g DM); NLT: number of leaves per tiller; LAR: leaf appearance rate (leaves/tiller/d); NL: number of leaves (leaves/pot); TPD: tiller population density (tillers/pot); FAR: forage accumulation rate (g DM/d); PHY: phyllochron (days/leaf).

Two-way dendrograms generated from hierarchical cluster analysis are illustrated as a heatmap (Figure 3).

Green color denotes variable (rows) with relatively greater corresponding content (classified among the strongest) in treatments (column). In contrast, gray color denotes variable with lesser content of the corresponding element (classified among the weaker). The heatmap indicates that Xaraés with 9 and 12 d N application timing after harvest are characterized by the group with greater values for TPD and PHY (classified among the strongest). Xaraés and Marandu with 0, 3, 6, 9 and 12 d N application timing after harvest are characterized by lesser values for height, FM, FAR, LM, NLT and LAR (classified among the weaker). Tanzânia with 6, 9 and 12 d N application timing after harvest is characterized by the greatest values for height, ILM, LM, FM and FAR (classified among the strongest) and lesser values for SM. Tanzânia with 0 and 3 d N fertilizer timing after harvest are characterized by greater values for LAR, NLT, FAR, FM, LM, LN, ITM and height (classified among the strongest) and lesser values for PHY and SM (classified among the weaker).

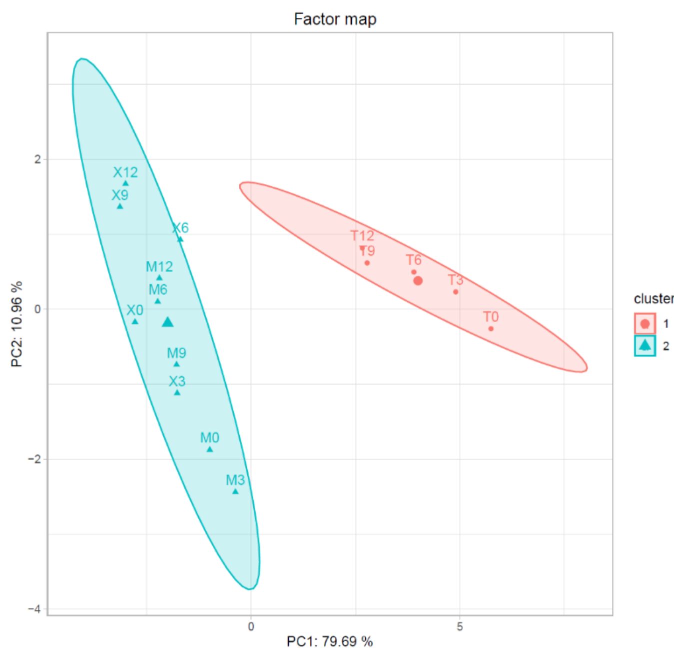

Figure 2. Factor map with combination of Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass, at different nitrogen application times (0, 3, 6, 9 and 12 days after harvest).

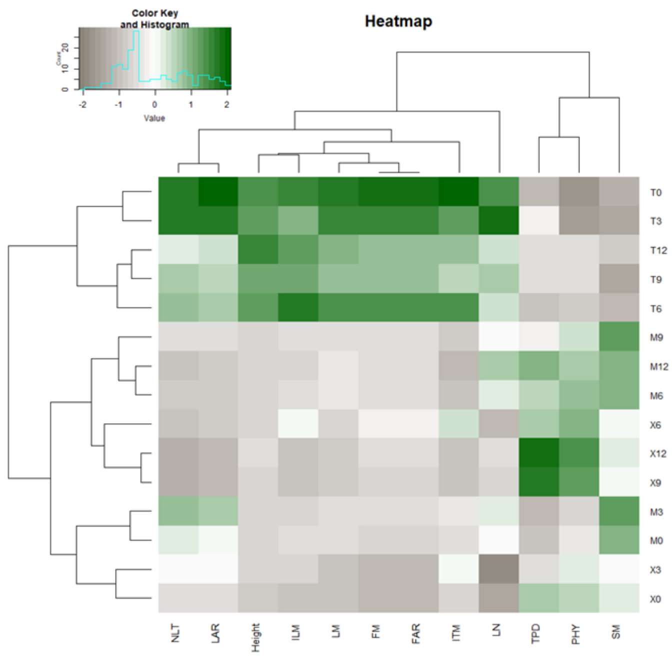

Figure 3. Heatmap analysis of plant responses in Xaraés (X) and Marandu (M) palisade grass and Tanzânia (T) guinea grass, at different nitrogen application times (0, 3, 6, 9 and 12 days after harvest).

Discussion

This study has provided interesting data on the differing responses of grasses from different genera, when N application after harvest is delayed. The results suggest that these different grasses need to be treated differently following harvesting, or at least will be affected differently by different post-harvest management, especially N application. As there was no effect of delayed application of N on forage mass for Xaraés and Marandu, it is likely shoot carbohydrate synthesis was not reduced (mainly leaf blades), which maintained root mass at similar levels among treatments (Tables 1 and 2). Roots are responsible for absorption of water and nutrients, as well as accumulating reserve carbohydrates during stress (Xiao and Jespersen 2019), including periods of low photosynthetic activity (after defoliation, water deficit and shading).

The increase in tillering without change in ITM, NLT and TPD highlights the phenotypic plasticity (Lopes et al. 2018) of Xaraés and Marandu, because even with modification in tillering, FM was maintained, reflecting a tiller size/density compensation (Sbrissia and Silva 2008).

Fertilizing Xaraés and Marandu with N shortly after harvesting provided fewer tillers with more leaves per tiller and therefore, heavier tillers. In contrast, when fertilizer application was delayed, there was an increase in TPD, with fewer leaves per tiller, resulting in lighter tillers. Higher TPD increases soil cover, which reduces the opportunity for weeds to appear and can increase the longevity of perennial pasture. Overall, weeds appear in areas with low tiller density and heavier tillers (Carvalho et al. 2016).

Marques et al. (2016) also observed a greater TPD in ‘Massai’ guinea grass (*Megathyrsus maximus* × *M. infestus* syn *Panicum maximum* × *P. infestum* cv. Massai) pastures when fertilized 7 days after harvesting. This phenomenon is known as tiller size/density compensation (Matthew et al. 1995), and has been reported in several forage species, such as: ‘Mombaça’ guinea grass (*Megathyrsus maximus* cv. Mombaça) (Alexandrino et al. 2011), oats (*Avena sativa*) and annual ryegrass (*Lolium perenne*) (Duchini et al. 2014), tifton 68 bermuda grass (*Cynodon nlemfuensis*) (Sbrissia et al. 2003) and Marandu palisade grass (Sbrissia and Silva 2008).

Besides tiller size/density compensation, delaying N fertilizer application influenced the growth pattern of Xaraés, as NLT declined as N application was delayed. This showed that although FAR and FM were not influenced by the timing of fertilizer application, as the delay increased, the emission of leaves was slower, but there was compensation with the larger TPD.

Tanzânia guinea grass forage mass, LM and plant height reduced as N application was delayed, along with number of leaves per pot and per tiller, but individual leaf mass did not alter. The reduction in leaf numbers was due to the increased PHY, which delayed leaf emission and resulted in fewer leaves per tiller and therefore reduced mass of individual tillers. Thus, Tanzânia was strongly influenced by N fertilizer application in the early days after harvest, which is different from the situation with Xaraés and Marandu. This suggested that fertilizer application for Tanzania must be carried out as soon as possible after harvesting. Grasses vary in their need for early application of N after harvest because of variation in organic reserve levels, which include N and carbohydrates accumulated in the base of the stem and roots (Pedreira et al. 2017).

Although these grasses were not strictly compared in the same experiment, this was a pot experiment in a glasshouse, which should minimize environmental differences. In addition, there is existing evidence that Tanzânia guinea grass has lower non-structural carbohydrate concentration (Soares Filho et al 2013) than Xaraés (Rodrigues et al. 2007) and Marandu (Alexandrino et al. 2008). Faria et al (2019) also suggested grasses with greater root starch concentration (non-structural total carbohydrate) are not disadvantaged by delaying N fertilizer application after harvesting as opposed to those with lower root starch concentration.

Although there was a 13% reduction in FM in Tanzânia with delay in N application, there was no effect on root mass (Table 3). Maintaining adequate root and residue masses improves the response potential of a grass under stress conditions (harvest, water stress, shading), because these organs are responsible for accumulation of reserve carbohydrates, which drives the regrowth under reduced photosynthetic activity (White 1973).

While delaying application of N fertilizer after harvest did not affect TPD of Tanzania, it did reduce individual tiller mass. In addition, there was a reduction in number of leaves per pot and per tiller as N fertilizer application was performed later, without altering individual leaf mass. Other studies reported a slight effect of the timing of fertilizer application on forage mass of Tanzânia guinea grass (Marques et al. 2016; Faria et al. 2019; Gomide et al. 2019).

While the longest delay (12 days after harvest) caused a reduction in number of leaves per tiller, it did increase the PHY. Increased PHY has been reported as being associated with N stress (Teixeira et al. 2014; Paciullo et al. 2017), which shows that delay in applying N after harvest may result in a short-term N deficiency. It is known that N reserves can influence the regrowth process (Schnyder and Viser 1999; Lehmeier et al. 2013); however, the physiological processes are still not well understood (Silva et al. 2015).

Forage accumulation rate, height, FM, ILM and ITM were grouped for Tanzânia, while PHY, TPD and SM were grouped for Xaraés and Marandu. For Xaraés and Marandu, individual tiller mass was inversely correlated with tiller population density, which was a reflection of an increase in PHY and reduction in LAR as application of N after harvest was delayed. The heatmap complemented the PCA and Cluster analysis results, in the differentiation between the forages and the timing of N application. There was an indication that tiller population density and PHY were greater for Xaraés and Marandu palisade grass if application of N after harvest was delayed by more than 6 days. In the case of Tanzânia guinea grass LAR, NLT, FAR, FM, ITM and LN were greatest if N was applied within 3 d after harvest.

Thus, grasses with low organic reserves or rapid growth potential demand rapid nutrient supply after harvest. Another hypothesis is that the reduction in forage production due to delayed fertilizer application will result in greater accumulation of nonstructural carbohydrates in the roots and stem base. This is a response reported when grasses are under flooding stress conditions (Ramos et al. 2011). In this case, the longer interval before N fertilizer application would promote nutritional stress due to the deficit of N reserves. Studies on N reserves in tropical grasses are scarce compared with studies in temperate forages (Avice et al. 1996; 1997).

This flexible response to fertilizer application several days after harvest in pastures like Xaraés and Marandu enables flexibility in management of grazing systems, mainly in intermittent stocking. A reduction in FM is avoided if a delay in fertilization is required, such as delay in fertilizer delivery, machinery breakdowns and/or maintenance and several other limitations that occur regularly on a farm.

Conclusion

In all grasses, there is an increase in PHY and reduction in NLT as N fertilizer application is delayed after harvest. While growth of Tanzânia guinea grass was reduced as fertilizer application was delayed, there was no effect on yield of Marandu and Xaraés palisade grass, since an increase in tillering compensated for any reduction in growth per tiller. Based on our results, N fertilizer application to Tanzânia guinea grass should be carried out as soon as possible after harvest, and certainly within 3 days, while fertilizing of *U. brizantha* cultivars may be carried out as late as 6–12 days after harvest without any negative impact. These findings should be tested in a field study before being recommended widely.

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(Note of the editors: All hyperlinks were verified 8 March 2021).

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