Nitrogen

Article

Effect of Time of Nitrogen Fertilization on Use of Root Reserves in *Megathyrsus maximus* Cultivars

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Abstract: Nitrogen is a very important nutrient in grass maintenance fertilization and therefore must be applied at the appropriate moment. The objective of this study was to identify the most responsive moment to nitrogen fertilization and to verify if root mass and the content of carbohydrates and nitrogen in roots influence the moment of fertilization in cultivars of *Megathyrsus maximus* (syn. *Panicum maximum*). This study was carried out simultaneously in a greenhouse using a completely randomized design, with sixteen treatments and five replications, in a 4×4 factorial design. The treatments consisted of four intervals between cultivar defoliation and nitrogen fertilization (0, 3, 6 and 9 days) and four *Megathyrsus maximus* cultivars, Mombasa, BRS Zuri, BRS Quenia and BRS Tamani, which were evaluated in five regrowth cycles. No difference in forage mass was observed among cultivars when fertilization was performed on days zero, three and nine after harvesting. On day nine, Mombasa showed a higher forage mass compared to BRS Tamani. Nitrogen content in the roots of Zuri decreased when fertilization was performed on the third day after defoliation, remaining constant in the other fertilization intervals. A linear reduction in root starch in BRS Zuri was observed, while in Mombasa cultivars, a linear increase was observed when fertilization was performed nine days after harvesting. Thus, nitrogen fertilization of BRS Tamani should be carried out closer to defoliation, while Mombasa, BRS Zuri and BRS Quenia can be fertilized up to nine days after harvesting, which results in greater flexibility regarding the moment of nitrogen fertilization.

Keywords: maintenance fertilization; root nitrogen; root starch

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1. Introduction

Nitrogen is the main macronutrient limiting tropical grass growth due to being the nutrient most extracted from soil by plants [1]. Nitrogen has an important role in metabolic pathways that result in protein synthesis and allow for the growth of structural organs in plants [2]. Thus, the absence of nitrogen in soil is widely known as the main reason for pasture degradation [3] and a reduction in corn productivity, which is also a forage utilized for silage [4–6].

However, for maximum forage growth, just the utilization of nitrogen as a fertilizer does not ensure maximum efficiency. In grazing systems where highly productive grasses are used, the requirements of nutrients for grasses such as *Megathyrsus maximus* are higher and an adjustment in the amount of fertilizer is needed. Studies have evaluated the dose of nitrogen that provides the best growth response in tropical grasses [7–9].

Although there is information regarding the time for applying nitrogen in pastures [10–14], it is important to better understand the physiological process involved, especially in grasses with high nutritional requirements, such as *Megathyrsus maximus* cultivars. For these cultivars, divergent results were reported regarding the time of nitrogen fertilization. While there was no observed effect of the time of nitrogen fertilization on the growth of *Megathyrsus maximus* cv. BRS Zuri [10] and *Megathyrsus maximus* cv. Paredão [14], there was a reduction in the forage mass of *Megathyrsus maximus* cv. Tanzânia [13] and *Megathyrsus maximus* cv. Tamani [14] as nitrogen fertilization was delayed. Another study evaluated the impact of three different fertilization times after harvesting (one, three and seven days) on *Megathyrsus maximus* × *Megathyrsus infestus* cv. Massai and observed effects only on leaf mass [11].

The hypothesis of this study was that the differences in the morphological and productive patterns of different *Megathyrsus maximus* cultivars would require the use of root reserves in different ways and the time in which the nitrogen is replaced in the soil could affect the use of the root reserves by these different cultivars. In this way, the objective of this study was to identify the effects of the time of nitrogen fertilization after harvesting on forage mass production, morphological characteristics, and root nitrogen and carbohydrate accumulation in BRS Tamani, BRS Quenia, Mombasa and Zuri cultivars.

2. Materials and Methods

2.1. Local and Experimental Design

This study was conducted in a greenhouse in Cuiaba, Brazil (15°35′56′′ S, 56°5′42′′ W and altitude of approximately 180 m), and Rondonopolis, Brazil (16°28′15′′ S, 54°38′08′′ W and altitude of approximately 227 m), from January to July 2019. The experimental design was completely randomized in a factorial scheme of 4 × 4. The treatments included four cultivars of *Megathyrsus maximus* (Jacq) (Mombasa, BRS Quenia, BRS Tamani and BRS Zuri) and four fertilization times after harvesting (0, 3, 6 and 9 days), with five replicates per treatment. Each experimental unit consisted of pots of 4.5 dm^3.

2.2. Soil and Sowing

The soil (Table 1) was collected from the 0–20 cm layer at an available area in each experimental site, sieved in a 4 mm screen to remove large particles, and transferred to pots. The pots were filled up with the soil, leaving approximately 5 cm at the top to avoid water flowing out upon irrigation. The soil pH was measured by adding 10 g of the soil sample into a beaker with a 25 mL 0.01 M $CaCl_2$ solution and letting it stand for one hour before measuring the pH [15]. The concentration of phosphorus (P) was determined using the Mehlich-1 procedure [16]. Potassium (K), calcium (Ca), magnesium (Mg) and aluminum (Al) were analyzed following the methods described by Teixeira [17]. Hydrogen (H) was analyzed following Campos [18]. Cation exchange capacity (CEC) was calculated as the sum of Ca^{2+}, Mg^{2+}, Na^+ and K^+ in $cmol_c\ kg^{-1}$. Base saturation (V) was calculated as V (%): $[(100 \times CEC)/(CEC + (H^+ + Al^{3+})]$. Aluminum saturation (M) was calculated as M (%): $[(100 \times Al^{3+})/(CEC + Al^{3+})]$. Sandy, silt and clay portions were measured as described by Donagemma [19]. The soil classification for sites 1 and 2 was Cambisol and Ferrasol, respectively [20]. For acidity correction, the base saturation was increased to 50% by incorporating dolomitic limestone with a total neutralizing value (TNV) of 99% for 30 days. During this period, the soil was kept at the maximum water holding capacity.

The establishment fertilization was carried out by applying 300 mg dm^{-3} of phosphorus in both experimental sites. Then, 20 wells were made on the soil surface in each pot with a 2 cm depth and two seeds were sown per well, totaling 40 seeds per pot. After plant emergence, thinning was performed to remove the excess plants, leaving five seedlings per pot.

When the plants reached 20 cm, another round of fertilization was performed. Because the soil was different in the experimental sites, fertilization was adjusted for each one. In the Cuiaba experimental site, was applied 50 and 25 mg dm^{-3} of nitrogen and

potassium in each pot, respectively. In the Rondonopolis experimental site, was applied 100 and 70 mg dm^{-3} of nitrogen and potassium in each pot, respectively. The doses utilized in Cuiaba were lower due to the higher salinization potential since it is a soil with a sandy texture.

Table 1. Granulometric and chemical composition of soil collected in Cuiaba, MT (experimental site 1), and Rondonopolis, MT (experimental site 2).

Soil	pH	P	K	Ca	Mg	Al	H	CEC [1]	V [2]	M [3]	Sand	Silt	Clay
	$CaCl_2$	mg dm^{-3}		cmolc dm^{-3}					%		g kg^{-1}		
Site 1	4.7	8.5	43	0.95	0.39	0.2	2.3	3.95	36.7	12.1	823	43	134
Site 2	6.0	3.4	119	2.3	2.0	0.0	1.7	6.3	73.0	0.0	425	150	425

[1] Cation exchange capacity; [2] base saturation; [3] aluminum saturation.

2.3. Standardization and Data Collection

When the plants reached 60 cm, an initial residual cutting was carried out for BRS Zuri and Mombasa at 30 cm [21] above the ground, while for BRS Tamani and BRS Quenia, the residual cutting height was 15 cm [22]. The differences in the residual cutting height adopted among cultivars was to maintain the residual leaf area and avoid decapitation of the apical meristem, providing adequate regrowth for each cultivar. After the initial cut, nitrogen and potassium fertilization was applied on days 0, 3, 6 and 9, starting from the first period of evaluation. In the Cuiaba experimental site, the doses of nitrogen and potassium applied for all cultivars were 100 and 50 mg dm^{-3}, respectively. In the Rondonopolis experimental site, the doses of nitrogen and potassium applied for all cultivars were 200 and 100 mg dm^{-3}, respectively. On the same day, the chlorophyll index was obtained on four leaves per pot, utilizing a chlorophyll meter ClorofiLOG® 1030 (Falker, Porto Alegre, Brazil).

Five rounds of sample harvesting were carried out with an average of 22 days between the cuts. At this time, the cultivars reached an average height of 55.04 cm for Mombasa, 52.44 cm for BRS Zuri, 39.89 cm for BRS Tamani and 45.87 cm for BRS Quenia. The number of tillers (TPD) was counted in each pot, and the grasses were harvested at the residual height. With the harvested material, the number of leaves (LN) was counted, and no morphological separation was needed because the material harvested contained just leaves.

The harvested material was dried in an air-forced oven at 55 ± 5 °C for 72 h, and weighed to determine the forage mass. The residual mass was similarly dried and weighed. In the last round of harvesting, the residual material, which was considered the remaining plant structure below the harvesting cutting height and above the ground, was harvested. After collecting the residual material, the soil containing the roots was removed from the pots for root measurements. The roots were washed with a high-pressure water machine in a 4 mm screen to allow for the removal of all of the soil residue. The roots were dried and weighed following the same procedure as the leaf measurement. After being dried, the roots were grounded in a Wiley mill using a 1 mm screen for carbohydrate and nitrogen analysis.

2.4. Root Carbohydrate and Nitrogen Analysis

The water-soluble carbohydrate (WSC) and starch concentrations were analyzed in the roots following the method by Antrona [23]. The total amounts of non-structural carbohydrates (TNSCs) were obtained by the sum of the WSCs and starch. Root nitrogen levels were analyzed according to the AOAC method [24].

2.5. Statistical Analysis

The data were analyzed using the general linear mixed model method, following the PROC MIXED procedure (SAS® Institute Inc., Cary, NC, USA). The timing of N fertilizer application was considered the fixed effect. Each regrowth cycle, the sites and the replications (pots) were considered the random effects. The orthogonal polynomial

contrast ($p < 0.05$) was used to evaluate the effects (linear or quadratic) of the timing of N fertilizer application.

3. Results

An interaction was observed between the time of nitrogen fertilization and the forage mass of the cultivars (Table 2). No difference in forage mass was observed among cultivars when fertilization was performed on days zero, three and six after harvesting (Table 3). Mombasa showed a higher ($p < 0.05$) forage mass compared to BRS Tamani when fertilization was applied nine days after harvesting; however, forage mass did not differ between BRS Quenia and BRS Zuri. A linear decrease in forage mass was observed in BRS Tamani on day nine compared to day zero (Table 3). An interaction was also observed in the chlorophyll index ($p < 0.05$) in which a higher index was observed for BRS Quenia for all fertilization times and a linear decrease was observed for BRS Zuri from day zero to day nine (Table 3).

Table 2. Productive, structural and morphological characteristics of *Megathyrsus maximus* cultivars fertilized at different intervals after harvesting.

Variable	Cultivars	Interval	Cultivars × Interval	SEM
Number of leaves (leaves pot^{-1})	<0.001	0.064	0.891	5.678
TPD (tillers pot^{-1})	<0.001	0.204	0.754	2.662
Forage mass (g pot^{-1})	0.002	0.015	0.043	0.536
Residual mass (g pot^{-1})	0.006	0.648	0.688	4.394
Root mass (g vaso^{-1})	0.845	0.723	0.876	1.868
Root nitrogen (g kg^{-1})	0.008	0.410	0.027	0.459
Root WSC (mg g^{-1})	<0.001	0.010	<0.001	1.136
Root starch (mg g^{-1})	0.095	0.136	<0.001	0.654
Root TNSC (mg g^{-1})	<0.001	0.002	<0.001	1.303
Chlorophyll index	<0.001	0.002	0.041	0.578

TPD: tiller population density; Root WSC: root water-soluble carbohydrate; Root TNSC: root total non-structural carbohydrate; SEM: standard error mean.

Table 3. Forage mass and chlorophyll index of *Megathyrsus maximus* cultivars fertilized at different times after harvesting.

	Days after Harvesting				*p*-Value [1]	
Cultivars	**0**	**3**	**6**	**9**	**L**	**Q**
			Forage Mass (g pot^{-1})			
Mombasa	14.57 A	14.77 A	14.27 A	14.03 A	0.353	0.662
BRS Quenia	13.33 A	13.59 A	13.97 A	12.99 AB	0.756	0.185
BRS Tamani	14.17 A	13.54 A	13.42 A	12.15 B	0.003	0.449
BRS Zuri	14.00 A	14.88 A	14.20 A	13.59 AB	0.376	0.120
			Chlorophyll index			
Mombasa	34.2 B	30.3 B	31.8 B	31.1 BC	0.136	0.165
BRS Quenia	39.5 A	36.2 A	37.7 A	36.5 A	0.147	0.364
BRS Tamani	33.4 B	33.0 AB	33.7 AB	33.3 AB	0.908	0.992
BRS Zuri	35.2 AB	35.1 A	32.3 B	28.8 C	<0.001	0.143

Letters followed by the same letter in the column do not differ according to Tukey's test ($p > 0.05$). [1] Orthogonal polynomial contrast: L = linear; Q = quadratic.

No effects of the timing of nitrogen fertilization ($p > 0.05$) were observed for the number of leaves and tillers and the residual and root mass (Figure 1). BRS Tamani had the highest number of leaves and tillers followed by BRS Quenia, but no differences were observed between BRS Zuri and Mombasa (Figure 1). Mombasa and BRS Zuri showed a higher residual mass compared to BRS Quenia and BRS Tamani.

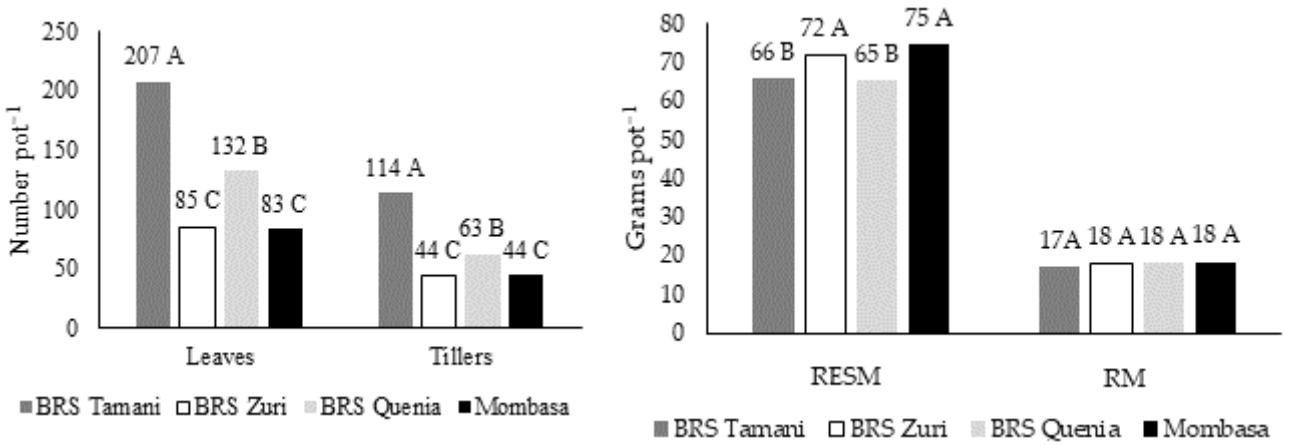

Figure 1. Number of leaves, tiller population density, and residual (RESM) and root mass (RM) of *Megathyrsus maximus* cultivars regardless of fertilization time. Capitalized letters followed by same letter within each variable do not differ according to Tukey's test ($p > 0.05$).

When nitrogen fertilization was performed on day zero, BRS Zuri showed the highest root nitrogen concentration, and no difference was observed among the cultivars Mombasa, BRS Quenia and BRS Tamani (Table 4). A quadratic reduction was observed in BRS Zuri in which a decrease was observed from day zero to day three and stabilizing posteriorly (Figure 2).

Table 4. Nitrogen, water-soluble carbohydrate, starch and total non-structural carbohydrate concentration in root of *Megathyrsus maximus* cultivars fertilized at different intervals after harvesting.

Grass	Days after Harvesting				*p*-Value [1]	
	0	3	6	9	L	Q
			Nitrogen (g kg^{-1})			
Mombasa	4.24 B	4.35 A	4.19 A	4.34 A	0.936	0.958
BRS Quenia	4.17 B	4.42 A	4.27 A	4.40 A	0.693	0.842
BRS Tamani	3.97 B	3.85 A	4.16 A	4.24 A	0.505	0.801
BRS Zuri	6.81 A	4.75 A	4.70 A	4.25 A	<0.001	0.034
			Water-soluble carbohydrates (mg g^{-1})			
Mombasa	11.26 B	8.96 AB	7.70 B	14.94 A	0.057	<0.001
BRS Quenia	8.57 B	6.28 B	9.23 B	10.32 AB	0.108	0.139
BRS Tamani	7.98 B	8.45 AB	10.79 B	5.91 C	0.447	0.020
BRS Zuri	16.80 A	12.08 A	17.62 A	12.81 B	0.209	0.967
			Starch (mg g^{-1})			
Mombasa	5.64 B	5.61 B	6.29 A	7.95 A	0.011	0.200
BRS Quenia	8.30 A	5.51 B	7.75 A	7.22 A	0.737	0.086
BRS Tamani	6.88 AB	6.32 AB	6.44 A	5.99 AB	0.387	0.936
BRS Zuri	8.60 A	8.11 A	7.47 A	4.81 B	<0.001	0.100
			Total non-structural carbohydrates (mg g^{-1})			
Mombasa	16.91 B	14.58 B	13.96 B	22.89 A	0.003	<0.001
BRS Quenia	18.87 B	11.80 B	16.98 B	17.55 B	0.217	0.032
BRS Tamani	14.87 B	14.77 B	17.24 B	11.91 C	0.273	0.046
BRS Zuri	25.41 A	20.20 A	25.10 A	17.63 B	0.002	0.386

Letters followed by the same letter in the column do not differ according to Tukey's test ($p > 0.05$). [1] Orthogonal polynomial contrast: L = linear; Q = quadratic.

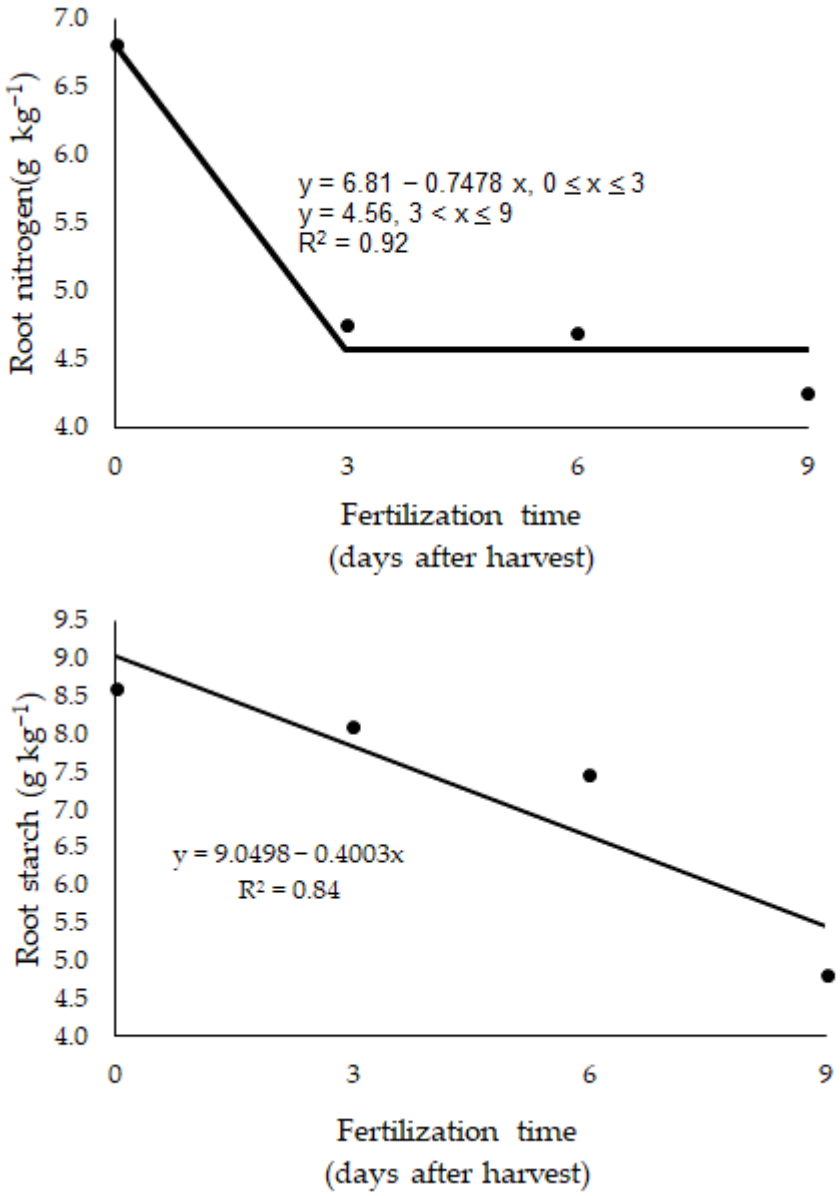

Figure 2. The nitrogen and starch content in the roots of *Megathyrsus maximus* cv. Zuri according to the time of fertilization.

A quadratic reduction in root WSC concentration in Mombasa was observed from day zero to day six, and an increase on day nine was seen (Table 4). In BRS Tamani, a quadratic increase was observed from day zero to day six followed by a decrease on day nine in root WSC concentration. A linear increase was observed in root starch content in Mombasa from day zero to day nine and a linear decrease in BRS Zuri was noted (Table 4). A decrease in the TNSC content was observed in BRS Quenia, BRS Tamani and BRS Zuri from day zero to day nine; however, in Mombasa, we observed an increase (Table 4).

4. Discussion

The only grass that had their forage mass affected by the fertilization time was BRS Tamani. The fertilization time effect on the forage mass produced by BRS Tamani is linked to the higher number of leaves and tillers observed for this cultivar, since younger tillers with less than five phytomers can use nitrogen translocating from older tillers [25]. Thus, given the high number of tillers, BRS Tamani has elevated nitrogen requirements right after harvesting to produce more leaves. The delay in fertilization did not affect the recycling

of nitrogen from harvesting to the day of fertilization since the chlorophyll index was the same between all fertilization intervals, demonstrating the non-occurrence of chlorosis.

These results demonstrate that with the exception of Tamani grass, *Megathyrsus maximus* cultivars do not need to be fertilized immediately after defoliation, since the delay in fertilization will not require a reduction in the stocking rate, as there was no effect on forage mass. This same effect was observed for Zuri [10] and Massai [11] cultivars, which are cultivars of *Megathyrsus maximus*, and also for cultivars of *Brachiaria brizantha* [13] and hybrids of *Brachiaria* spp. [26]. It has been observed that the diversity of responses regarding the timing of fertilization does not only depend on the grass, but also on the fertilization method used [27].

Furthermore, delayed fertilization does not accelerate pasture degradation, as tillering and the number of leaves were not affected by the fertilization time. Tillering is important for soil coverage, weed suppression and pasture longevity [28,29]. Root mass was also not altered by the time of fertilization, which demonstrates that even though there was a change in nitrogen and sugar content in some cultivars (Table 4), there was not enough nutritional stress to reduce root mass. A reduction in root mass can be seen under various stresses: nutritional deficits [30], water stress [31], grazing intensity [32] and others.

The time of fertilization seems to not have an effect on nitrogen and starch in the roots of BRS Tamani, since no changes were observed when fertilization was performed after nine days. In this case, this grass did not endure nutritional stress when delaying fertilization because the grass could recover its nutrient reserves until the next round of harvesting, which happened 22 days after. Thus, it is not possible to affirm that the reduction in forage mass observed in BRS Tamani was due to the shortage in nitrogen reserves, which are important for grass re-establishment after harvesting [33].

In this way, due to BRS Tamani being a grass with elevated tiller numbers, it is possible that this grass had prioritized tillering instead of tiller density. Thus, part of the nitrogen required for tissue synthesis was utilized for cytokinin biosynthesis, which is a phytohormone relevant to gem activation. In light of this, further studies must be performed to evaluate the association between nitrogen fertilization time and the use of exogenous cytokinin to confirm this hypothesis. In wheat, the same tiller number was observed when isolated nitrogen fertilization was performed compared to when nitrogen fertilization was associated with exogenous cytokinin; however, more shoot biomass and root mass were observed when nitrogen fertilization was applied with the use of cytokinin [34].

Although fertilization time did not affect the forage mass of BRS Zuri, it was possible to observe that by waiting three days after harvesting for fertilization, the nitrogen reserves in the roots decreased; however, this decrease did not have an impact on the forage mass of this grass. In this way, BRS Zuri utilized its root reserves of nitrogen to allow for the continuous growth of its forage mass, and it is probable that the rate of uptake of nitrogen from soil by the roots is not the same as the transport of nitrogen to the shoots for regrowth, leading to a negative balance in nitrogen in the roots [35].

The rate at which the grass can regrow the shoot mass after harvesting depends on a diversity of physiological mechanisms, especially the accumulation and absorption of nitrogen in the roots [36]. Nitrogen has an important role in grass re-establishment because it is a constituent of chlorophyll, proteins and enzymes which participate in the photosynthesis process, being the main process responsible for energy production by plants [37].

Furthermore, regarding the decrease we observed in the starch content in the roots with the increase in water-soluble carbohydrates, it is possible that the carbon coming from the starch breakdown was utilized for plant regrowth, and because of that, there was no impairment in the forage mass of BRS Zuri. In a nutritional stress condition, the starch can be cleaved into soluble sugar which will be used in the respiration process as a source of energy for regrowth [38,39].

On the other hand, starch synthesis starts to be carried out when the accumulation of sucrose exceeds the storage capacity of the leaf or when the demand of the tissues

considered is greater [38]. Therefore, the interval between the last round of fertilization (nine days) of BRS Zuri and the next harvesting round was not enough to restore the starch content, which resulted in a greater decrease in the content of this reserved carbohydrate.

Similarly, the interval between harvesting and fertilization did not influence the forage mass of Mombasa and BRS Quenia. These results demonstrate that Mombasa and BRS Quenia are more efficient in reconstituting the starch content in their roots compared to BRS Zuri. Mombasa was the only grass in which there was an increase in starch in the roots. The accumulation of non-structural carbohydrates occurs when the synthesis of photoassimilates is greater than the amount used by plants for growth and respiration [40].

Mombasa and BRS Tamani were the only cultivars in which the time of fertilization influenced the levels of WSCs. Sucrose is the main substrate for respiration, translocated by the phloem, and when plants are subjected to some stress factors, sucrose will be the first source of energy to be used [37]. In light of this, it was observed that from the sixth to the ninth day, there was a 45% reduction in the content of WSCs in the roots of BRS Tamani. This is a result of lower photosynthetic activity since there was a reduction in forage mass, which was composed solely of leaf blades, which are the main photosynthetic structure.

Therefore, BRS Zuri, Mombasa and BRS Quenia cultivars showed flexibility regarding the time of fertilization; however, physiological stress was observed for BRS Zuri, which may demonstrate the adaptation of this grass to the delay in replacing nitrogen in the soil, which demands further studies to evaluate these hypotheses.

The results indicate that fertilization can be carried out within a period of up to nine days without a decrease in the forage production of BRS Zuri, Mombasa and BRS Quenia cultivars, which facilitates the management of fertilization in systems with rotational stocking. However, for BRS Tamani, nitrogen fertilization should be carried out as soon as possible after harvesting to avoid losses in forage mass. Future studies should subject grasses to stress (water, nutritional or cutting height) and apply different times of fertilization to verify whether the changes that stress causes in the root system [26–28] can compromise the response to fertilization.

5. Conclusions

Fertilization time, in general, does not affect the forage mass of *Megathyrsus maximus* cultivars. The only cultivar that shows a reduction in forage mass with delayed fertilization is Tamani grass. In none of the *Megathyrsus maximus* cultivars did the timing of fertilization affect root mass and the number of tillers and leaves, which demonstrates that delaying fertilization does not cause nutritional stress and does not cause pasture degradation. Therefore, Mombasa, BRS Zuri and BRS Quenia cultivars showed more flexibility regarding the time of fertilization, allowing fertilization with nitrogen to be performed up to nine days after harvesting, while BRS Tamani should be fertilized immediately after harvesting to avoid a decrease in forage mass. The changes in the forage mass of Tamani grass due to the timing of fertilization are not explained by the nitrogen and sugar content in the roots.

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