

Marandu palisadegrass growth and nutrient accumulation as affect by nitrogen and sulfur fertilizations

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Abstract

Nitrogen fertilization provides structural and productive changes in forage grasses, but there is a need to know the demand for sulfur, mainly in soil with low availability of this nutrient. The objective was to evaluate the effects of nitrogen and sulfur applications on growth attributes and nutrient concentration in Marandu palisadegrass (*Brachiaria brizantha* cv. Marandu). Experiment was carried out under greenhouse conditions during spring season, with an Entisol that exhibited very low organic matter content and low availability of sulfate-sulfur. Combinations of five nitrogen rates (0, 100, 200, 300 and 400 mg dm⁻³) and five sulfur rates (0, 10, 20, 30 and 40 mg dm⁻³) were used in a fractionated 5² factorial, with four replications. Number of leaves, number of tillers, leaf area, dry matter production and nitrogen concentration in shoot of the grass showed significant responses to nitrogen fertilization at the first harvest. Sulfur concentration in shoots sampled at the first harvest depended on both nitrogen and sulfur fertilizations. To maximize the response variables in the following two harvests it was necessary to supply sulfur in the fertilization. At the second and third harvests, average dry matter production of the forage grass with no nitrogen or no sulfur supply was 21 times smaller than that found with nitrogen rate of 300 mg dm⁻³ and sulfur rate of 30 mg dm⁻³. Structural variables and dry matter production of grass showed the same pattern of response in each growth period.

Keywords: *Brachiaria brizantha*; Dry matter; Leaf area; Number of leaves; Tiller.

Introduction

Forage grass production is intrinsically related to the continuous appearance of tillers and leaf development and growth processes (De Bona and Monteiro, 2010a), which is responsible for the restoration of leaf area and increase in canopy photosynthetic capacity after mechanical harvesting or grazing. The processes of leaf formation and development are fundamental to plant growth and dry matter production, since leaves are essential to photosynthesis, which is the starting point to the formation of new tissues (Lemaire and Chapman, 1996). In practice, number of leaves affects the harvest age. Appearance of tillers helps pasture covers the soil in a short time, decreasing the chance of invasion by weed especially during pasture establishment. The production system and maximization of biomass production of forages depend on the environment and management (Lopes et al., 2013).

In the tropics, usually there are limitations in soil fertility to plant nutrition. Therefore, nutrient replacement to the soil by fertilization can increase the pasture production, especially through positive response to nitrogen fertilization (Xia and Wan, 2008), for its positive effect in biomass flow (Duru and Ducrocq, 2000) and nutritional value of forage (Andrade et al., 2003). Due to their morphophysiological characteristics, such as plant architecture and photosynthetic efficiency, tropical forage grasses respond positively to high nitrogen rates (Cantarutti et al., 2002). The optimal nitrogen fertilizer rate is laborious to be defined, because there are differences among plants requirements, field management and soil conditions.

Plant nutrition can affect soil-plant interactions. Nitrogen and sulfur are important, because they participate in both

systems simultaneously. In the soil they are constituents of organic matter (Stevenson, 1985) and in the plant they are components of amino acids (Crawford et al., 2000).

Supply of nutrients in amounts and proportions, particularly nitrogen and sulfur, are important for the pasture production, because nitrogen and sulfur mineralization from soil organic matter usually does not meet the demand of forage grasses with high yield potential (Fagundes et al., 2006).

Studies have demonstrated that the use of sulfur in fertilization simultaneously to nitrogen can increase the amount of these nutrients removed from soil and increase dry matter production of grasses (Mathot et al., 2008) because it is related to nitrogen metabolism (Crawford et al., 2000). The interactions N × S have been researched in many crops such as wheat (*Triticum aestivum* L.) (Wooding et al., 2000), oat (*Avena sativa* L.) (Wang et al., 2002) and corn (*Zea mays*) (Chen et al., 2008).

Therefore, this research aimed to evaluate the effects of simultaneous nitrogen and sulfur applications on growth attributes and nutrient concentration in the shoots of Marandu palisadegrass, in three growth periods when the combinations of these nutrients were applied to a soil with low availability of sulfur sulfate (2 for 6 mg dm⁻³) and organic matter (9,5 mg dm⁻³).

Results

Plant production

Nitrogen rates increased the number of tillers per pot at the first harvest of the grass (Fig. 1a), and the maximum number

of tillers occurred at the nitrogen rate of 307 mg dm⁻³. Addition of this nitrogen rate increased in 71% the number of tillers, compared to the non-supply of this nutrient. Combination of nitrogen and sulfur rates was significant to the tillering at the second and third harvests of grass (Fig. 1b and 1c). Most effective combinations for maximum tillering were respectively of 358 and 29 mg dm⁻³ in second harvest and 299 and 35 mg dm⁻³ for third harvest of Marandu palisadegrass.

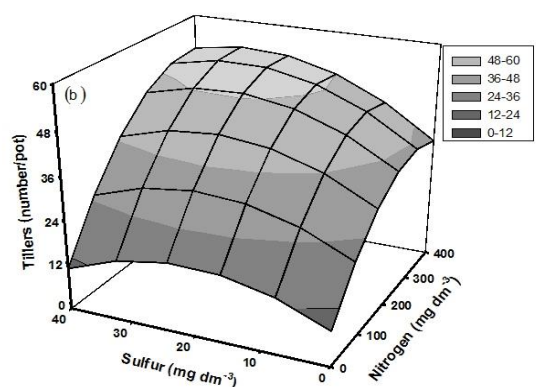
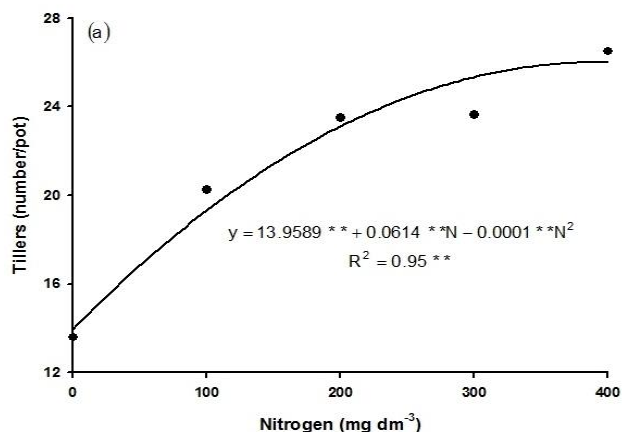
Nitrogen supply of 300 mg dm⁻³ without sulfur supply led to 34 and 53 tillers, in average, at second and third harvests, respectively. However, by adding 30 mg dm⁻³ of sulfur, number of tillers increased to 50 and 87 in second and third harvests, respectively, which represents an increase of 47 and 64%, compared to when only nitrogen had been applied.

Number of leaves of forage grass per pot, at the first harvest, was significantly changed by nitrogen rates, with highest number found at the rates of 297 mg dm⁻³ (Fig. 2a). An interaction nitrogen rate × sulfur rates was significant to the number of leaves at the second and third harvests of grass (Fig. 2b and 2c).

Nitrogen and sulfur rates resulting the highest number of leaves were 363 and 29 mg dm⁻³ (second harvest), and 300 and 36 mg dm⁻³ (third harvest). Higher number of leaves was observed in second and third harvests of forage grass than at the first one. In order to obtain a large number of expanded leaves both in second harvest (123 leaves in five plants) and in third harvest (161 leaves in five plants) of this forage grass, high rates of both nitrogen and sulfur were demanded. Nitrogen fertilization influenced positively the leaf area of Marandu palisadegrass at the first harvest (Fig. 3a). Differently from first harvest of the forage grass, significance in leaf area was observed for nitrogen rates × sulfur rates interaction at the second and third harvests (Fig. 3b and 3c). At the second harvest, nitrogen and sulfur rates applied did not result in maximum leaf area in the grass. At the third harvest, maximum leaf area was obtained when nitrogen rate of 371.6 mg dm⁻³ was combined with a sulfur rate of 39.5 mg dm⁻³ (9.5:1 ratio between these rates).

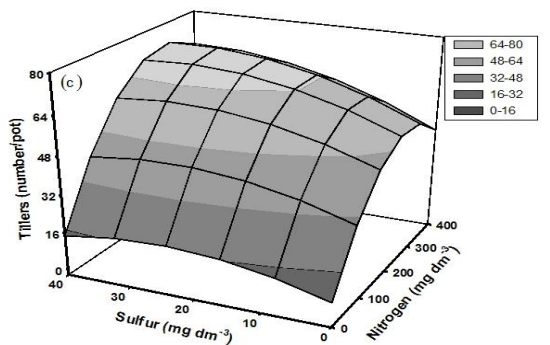
At the first harvest, shoot dry matter production responded significantly to nitrogen supply (Fig. 4a), and to nitrogen rates × sulfur rates in second and third harvest (Fig. 4b and 4c). Maximum dry matter production occurred for the nitrogen rate of 387 mg dm⁻³ in the first harvest, and for the nitrogen × sulfur combinations of 345 and 31 mg dm⁻³ and 317 and 32 mg dm⁻³ at the second and third harvests, respectively. For maximum shoot dry matter production of forage grass at the second and third harvests, nitrogen:sulfur ratios in fertilizations were equal to 11.1:1 and 9.9:1, respectively.

Increments in number of tillers and leaf area (Fig. 1 and 3) probably increased nutritional demand for sulfur from the second growth period on, because the effect of sulfur supply to the grass fertilized with nitrogen was observed from that period onwards. Soil, even with very low sulfur content (2.6 mg kg⁻¹ of sulfate-sulfur extracted with calcium phosphate solution) was not a limitation to the first growth of the grass (Fig. 4). At the second and third harvests, average dry matter production of the forage grass with no nitrogen or no sulfur supply was 21 times smaller than that found with nitrogen rate of 300 mg dm⁻³ and sulfur rate of 30 mg dm⁻³. Structural variables (leaf area, number of tiller and number of leaf) and dry matter production of grass showed the same pattern of response in each growth period. It was observed a quadratic response for the applied nitrogen rates, at the first harvest, and significance of nitrogen rates × sulfur rates interaction at the second and third harvests.



$$y = 8.0583 ** + 0.1754 **N - 0.0003 **N^2 + 0.0009 **NS + 0.9125 **S - 0.0217 **S^2$$

$$R^2 = 0.87 **$$



$$y = 7.3102 + 0.3309 **N - 0.0006 **N^2 + 0.0011 **NS + 0.8384 **S - 0.0017 **S^2$$

$$R^2 = 0.87 **$$

Fig 1. Number of tillers of Marandu palisadegrass related to nitrogen at the first harvest (a) and related to the combinations of nitrogen and sulfur rates at the second (b) and third harvests (c).

Nutrient accumulated by plants

Nitrogen accumulated in shoots during first growth period responded positively to the nitrogen supply, while the interaction nitrogen rates × sulfur rates was significant at second and third growth periods. For accumulated sulfur in grass tissues, interaction of nitrogen × sulfur rates was

significant in each of the three harvests (Table 1 and Table 2).

Low contents of nitrate and ammonium, and sulfur-sulfate in initial soil sample reflected in the accumulated amounts of these nutrients by the plant, especially for plants with no nitrogen and sulfur supply (Table 1). For the first growth period of grass, in spite of low contents of these nutrients in soil after liming and organic matter mineralization, plants were well developed especially regarding to sulfur demand, which was low in that period, justifying the lack of response in structural variables and shoot dry matter production (Fig. 1, 2, 3 and 4). In the absence of nitrogen and sulfur supply, amount of these nutrients accumulated by the forage grass diminished from one harvest to the other, due to depletion of these nutrients availability in the soil. From second growth period onwards, need of nitrogen and sulfur supply was evident, justifying the plant response in structural variables and shoot dry matter production at the second and third growth periods to the simultaneous nitrogen and sulfur additions (Fig. 1, 2, 3 and 4). As nitrogen demand increased, sulfur demand also increased, resulting in high accumulation of these nutrients in the shoots.

High nitrogen rates combined with high sulfur rates provided increase in accumulated nitrogen and sulfur by Marandu palisadegrass at the second and third harvests. Combination of 400 mg dm⁻³ of nitrogen with 0 mg dm⁻³ of sulfur resulted in shoots accumulation of nitrogen of 449.0 and 235.7 mg/pot and accumulated sulfur of 7.2 e 3.6 mg/pot, while in the combination of 400 mg dm⁻³ of nitrogen with 40 mg dm⁻³ of sulfur, the accumulated nitrogen was 791.2 and 924.1 mg/pot and sulfur was 31.3 and 41.9 mg/pot at the second and third growth periods, respectively.

Discussion

The positive response in plant tillering to the nitrogen fertilization is associated with the stimulus of nitrogen to plant cells growth and multiplication, since this nutrient is a component of cellular proteins and nucleic acids (Oliveira et al., 2007). Garcez Neto et al. (2002) found significant effect of nitrogen supply in the number of tillers (21% increase the tiller population density) of Mombaça guineagrass (*Panicum maximum* cv. Mombaça). According to these authors, tillering in grasses is a determining structural feature in morphogenic plasticity of forage grass, influenced by combinations of nutritional, environmental and management factors. Rodrigues et al. (2008) found influence of nitrogen fertilization in the number of tillers of Marandu palisadegrass in two soils, recently fertilized and unfertilized. For maximum tillering, nitrogen supply of 184 and 161 mg kg⁻¹ were necessary for the former and the latter, respectively.

Increase in number of tillers from first to second harvests of Marandu palisadegrass took place because plant at the initial growth stage concentrates most of its energy in establishment, forming root and shoot systems. On the other hand, from second and third growth periods, plant was established, with a high volume of roots, and had the capacity to absorb more nutrients than plants at the initial growth stage. In addition, according to Langer (1974), effect of first harvest resulted in basal buds development and apical dormancy breaking, creating higher number of tillers in the following growth stages.

Increase in number of leaves can be explained by the supply of nitrogen rates that stimulate the formation of new tissues (Oliveira et al., 2007) and under nitrogen fertilization plants anticipated senescence, that is, showed higher tissue flow (appearance and death of tissues).

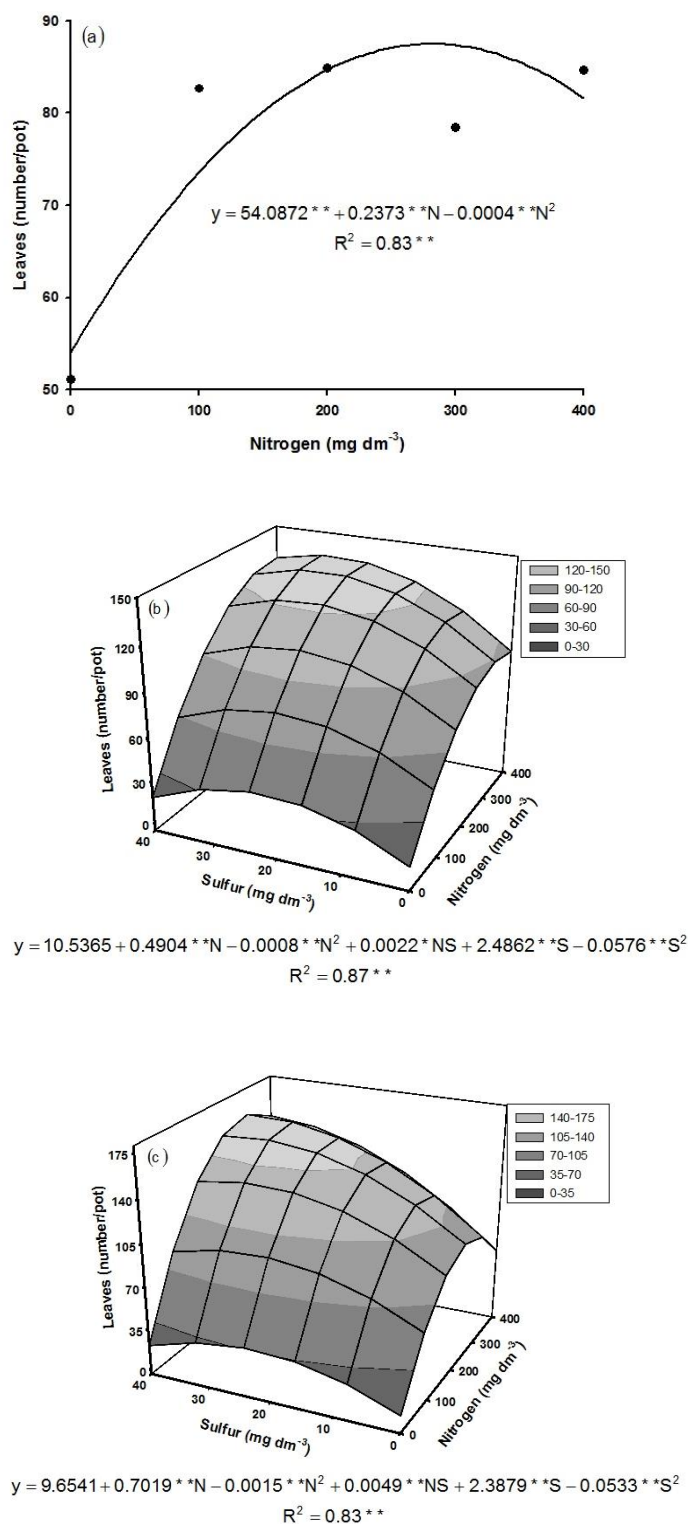


Fig 2. Number of leaves of Marandu palisadegrass related to nitrogen at the first harvest (a) and related to the combinations of nitrogen and sulfur rates at the second (b) and third harvests (c).

Table 1. Nitrogen and sulfur accumulated in shoots of Marandu palisadegrass as related to the combinations of nitrogen and sulfur rates at each plant harvest.

N x S	Nitrogen			Sulfur		
	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest
	-----mg/pot-----					
N ₀ S ₀	78.7 e	21.6 e	12.6 f	8.9 c	1.4 f	0.8 d
N ₀ S ₂₀	90.4 e	19.1 e	14.6 f	8.3 c	1.5 f	0.8 d
N ₀ S ₄₀	72.8 e	24.3 e	10.9 f	7.5 c	2.0 f	1.1 d
N ₁₀₀ S ₁₀	234.3 de	92.7 d	243.2 e	22.1 bc	9.8 ef	16.4 c
N ₁₀₀ S ₃₀	260.4 cde	244.2 d	254.1 e	27.7 ab	11.4 de	19.8 bc
N ₂₀₀ S ₀	369.9 bcd	277.5 d	133.3 ef	33.7 ab	6.4 ef	2.7 d
N ₂₀₀ S ₂₀	454.9 abc	430.9 c	474.7 d	36.5 ab	19.8 cd	28.8 b
N ₂₀₀ S ₄₀	450.5 abc	509.9 bc	460.5 d	38.8 a	21.2 bc	28.4 b
N ₃₀₀ S ₁₀	482.2 ab	577.7 bc	597.8 cd	32.0 ab	19.3 cd	24.8 bc
N ₃₀₀ S ₃₀	501.8 ab	638.6 b	688.2 bc	33.6 ab	29.7 a	38.3 a
N ₄₀₀ S ₀	556.9 ab	449.0 c	235.7 e	39.1 a	7.2 ef	3.6 d
N ₄₀₀ S ₂₀	606.1 a	829.4 a	775.3 ab	29.2 ab	29.2 ab	38.6 a
N ₄₀₀ S ₄₀	479.0 ab	791.2 a	924.1 a	25.2 ab	31.3 a	41.9 a

Means followed by the same letters, in each column, do not differ statistically by the Tukey test at 5% level.

Table 2. Regression equations based on nitrogen and sulfur accumulations (acc) in shoots of Marandu palisadegrass as related to nitrogen and sulfur rates at each plant harvest.

Response variable	Regression equations	R ²
	-----First harvest-----	
N acc	$y = 76.426 + 2.158N - 0.002N^2$	0.84**
S acc	$y = 6.938 + 0.226N - 0.0004N^2 - 0.0008NS - 0.089S + 0.005S^2$	0.76*
	-----Second harvest-----	
N acc	$y = 22.542 + 1.757N - 0.001N^2 + 0.020NS + 8.047S - 0.189S^2$	0.94**
S acc	$y = 1.074 + 0.063N - 0.0001N^2 + 0.002NS + 0.567S - 0.014S^2$	0.90**
	-----Third harvest-----	
N acc	$y = 12.633 + 1.247N - 0.001N^2 + 0.042NS + 13.513S - 0.357S^2$	0.93**
S acc	$y = 3.245 + 0.083N - 0.0001N^2 + 0.002NS - 1.096S - 0.026S^2$	0.92**

** significant at 1% level.

The balance established between nitrogen and sulfur in forage grass, maximized the leaf production, since the combinations of high nitrogen rates and no sulfur supply usually resulted in plant nutritional imbalance (Dijkshoorn and van Wijk, 1967). The combined nitrogen and sulfur supply influence in plant production can be explained by interdependence of biochemical routes in the assimilation of these nutrients (Crawford et al., 2000).

The increase in number of leaves can be related to higher number of tillers emitted during plant regrowth (at the second and third growth periods), since the number of leaves per tiller is relatively constant. In this context, larger number of leaves and tillers are important to a proper pasture yield, which conducts to a recommendation that a high nitrogen supply to plants must be combined with appropriate sulfur supply, in agreement with Mattos and Monteiro (2003).

Martuscello et al. (2005) found increases of even 130% in leaf area of Xaraés palisadegrass (*Brachiaria brizantha* cv. Xaraés) after applying a nitrogen rate of 120 mg dm⁻³, compared to the no nitrogen supply. Batista and Monteiro (2008) found increase in leaf area of Marandu palisadegrass when applying nitrogen and sulfur rates, with maximum leaf area obtained at a 7:1 ratio between nitrogen and sulfur rates, at the second harvest.

Leaf area of Marandu palisadegrass at the first harvest was smaller than at the second and third harvests, because the energy used for root system formation was much smaller after the first growth period and consequently more energy was available for shoot growth. Increase in leaf area of forage grass lead to higher photosynthetic efficiency (Parsons

et al., 1983), as it increased light interception area (Akmal and Janssens, 2004) and was a key factor for pasture yield. Nitrogen fertilization promotes direct increase in photosynthetic leaf area (Nabinger, 2001).

Leaf area is responsible for sunlight capture in a way that a larger leaf area allows higher exposition to sunlight. Chapman and Lemaire (1993) pointed out that nitrogen supply affects the leaf elongation, resulting in larger leaf area. In addition, nitrogen is a driving factor in the processes of plant growth and development, allowing increases in biomass due to increase in carbon fixation (Nabinger, 2001). Thus, the larger energy capture area may result in higher biomass accumulation.

For pastures at establishment, the production depends on nitrogen supply. However, when seeking to intensify pasture use, by applying nitrogen fertilizers, there is the need to meet the sulfur demand of the forage grass in soils with low sulfur content. The results of dry matter production by Marandu palisadegrass are in agreement with those reported by Mattos and Monteiro (2003) and Batista and Monteiro (2008), indicating that nitrogen increment leads to the higher sulfur demand in order to maximize yield responses of *Brachiaria decumbens* and *Brachiaria brizantha*, respectively. Besides the responses of the forage grass to sulfur, when fertilized with nitrogen, the application of sulfur may influence the efficiency of nitrogen use by the grass.

Positive responses in forage grass to sulfur application combined to nitrogen application was reported by Phillips and Sabbe (1994), when studying the dry matter production

of Coastal Bermuda grass (*Cynodon dactylon*) cultivated in soils poor in sulfur and organic matter.

De Bona and Monteiro (2010b), in an Entisol with high organic matter content and a sulfur-sulfate content 2.8 times higher than the one used in this experiment, did not obtain responses to the sulfur application in first growth period of Marandu palisadegrass, in terms of dry matter production, number of tillers and leaf area. This confirms the low sulfur demand by this forage grass during the establishment stage. In agreement with the results presented here, those authors also found that, starting at second growth period of the grass, addition of nitrogen to soil may have facilitated the activation of meristematic tissues and the increases in photosynthetic and light interception areas. In other words, it may have provided increase in absorbed amount of nutrients, their translocation, water uptake and consequently higher dry matter production, which explains the increase in nitrogen and sulfur demand by the Marandu palisadegrass after first growth period.

De Bona and Monteiro (2010b) found that nitrogen rates × sulfur rates interaction was significant to the accumulated nitrogen and sulfur in three harvests of forage grass. However, high nitrogen rates combined with low sulfur rates resulted in very low accumulated nitrogen in the shoots.

Although the soil presented low contents of organic matter (9.5 g dm⁻³) and sulfur-sulfate (2.6 mg dm⁻³), Marandu palisadegrass in the establishment stage only responded to nitrogen supply. In its following growth period, sulfur and nitrogen applications were demanded. Batista and Monteiro (2008) and De Bona and Monteiro (2010b) obtained similar results when they studied the same forage grass in soils with organic matter contents of 11.6 g dm⁻³ and 32.1 g dm⁻³ and sulfur-sulfate contents of 7.3 mg dm⁻³ and 7.4 mg dm⁻³, respectively. This result indicates that simultaneous addition of nitrogen and sulfur in a balanced way is necessary to maximize the production of this forage grass. Thereby, organic matter and sulfur-sulfate contents in a soil did not indicate precisely the needs of nitrogen and sulfur supply for the establishment of Marandu palisadegrass. For conditioning the response to nitrogen by the grass, sulfur must be considered in pasture fertilization after the initial establishment stage of forage grass.

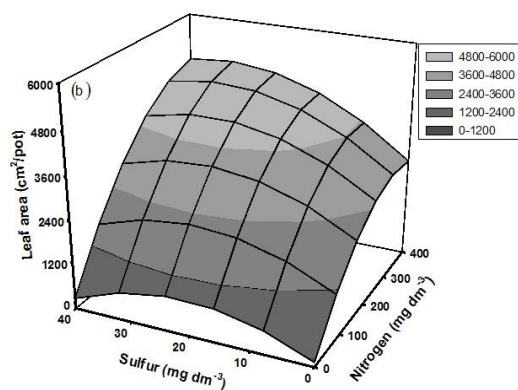
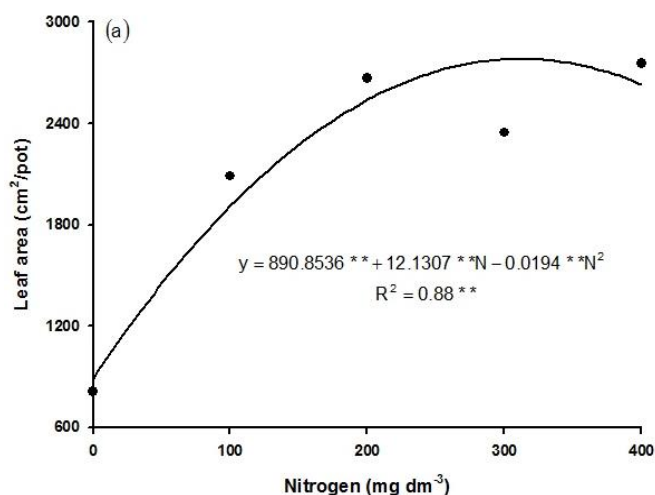
Material and Methods

Study site

This study was conducted under greenhouse conditions at Piracicaba, São Paulo State Brazil (22°43' S, 47°38' W), during the spring season. The average air temperature in the greenhouse during the experimental period was approximately 33°C. Marandu palisadegrass was chosen as representative of the *Brachiaria* genus, because this grass occupies approximately 95 million ha of agricultural land in Brazil.

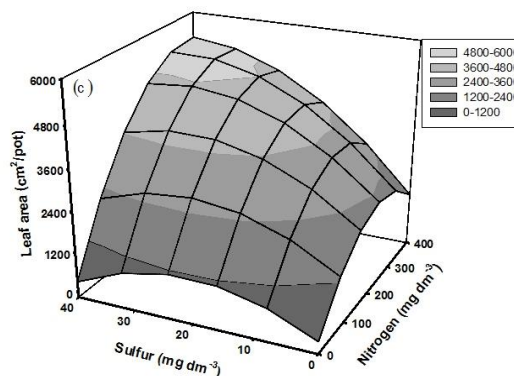
Soil characteristics

Sample of a soil classified as an Entisol were collected at 0-20 cm of depth, air-dried, sieved to pass through a 4 mm screen and mixed. Soil chemical characteristics showed the following results (Silva et al., 2006): P resin= 3 mg



$$y = 323.257 + 16.8443 * N - 0.0252 * N^2 + 0.1170 * NS + 83.6533 * S - 1.9868 * S^2$$

$$R^2 = 0.88 **$$



$$y = 288.0572 + 17.2971 * N - 0.0357 * N^2 + 0.2350 * NS + 95.5757 * S - 2.3178 * S^2$$

$$R^2 = 0.85 **$$

Fig 3. Leaf area of Marandu palisadegrass related to nitrogen at the first harvest (a) and related to the combinations of nitrogen and sulfur rates at the second (b) and third harvests (c).

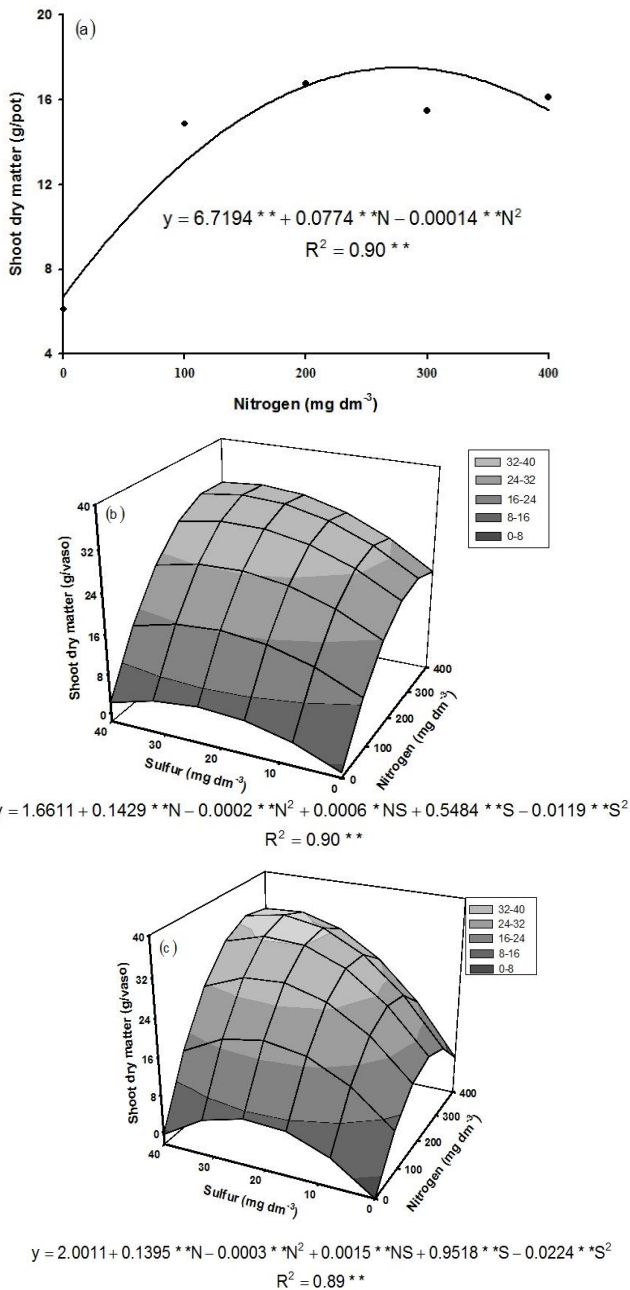


Fig 4. Shoot dry matter production of Marandu palisadegrass related to nitrogen at the first harvest (a) and related to the combinations of nitrogen and sulfur rates at the second (b) and third harvests (c).

dm^{-3} ; $\text{OM} = 9.5 \text{ g dm}^{-3}$; $\text{pH in CaCl}_2 = 4.0$; $\text{K}^+ = 2.3 \text{ mmol}_c \text{ dm}^{-3}$; $\text{Ca}^{2+} = 6 \text{ mmol}_c \text{ dm}^{-3}$; $\text{Mg}^{2+} = 2 \text{ mmol}_c \text{ dm}^{-3}$; $\text{Al}^{3+} = 8 \text{ mmol}_c \text{ dm}^{-3}$; $\text{H+Al} = 40 \text{ mmol}_c \text{ dm}^{-3}$; $\text{CEC at pH 7.0} = 50.3 \text{ mmol}_c \text{ dm}^{-3}$; $\text{sum of bases} = 10.3 \text{ mmol}_c \text{ dm}^{-3}$; $\text{base saturation} = 20\%$; $\text{S-SO}_4^{2-} = 2.6 \text{ mg dm}^{-3}$; $\text{total-N} = 400 \text{ mg dm}^{-3}$. Soil texture showed 75.1, 126.3 and 798.6 g dm^{-3} of clay, silt and sand, respectively.

Soil fertilizations

Liming to achieve 50% base saturation was done by using calcium carbonate and magnesium carbonate in a 1.5:1 ratio. Soil moisture was maintained at 70% of soil retention capacity with deionized water for incubation during 35 days.

Combinations of five nitrogen rates (0, 100, 200, 300 and 400 mg dm^{-3}) with five rates of sulfur (0, 10, 20, 30 and 40 mg dm^{-3}) were studied in fractionated 5^2 factorial, according to Littell and Mott (1975). The thirteen combinations between nitrogen and sulfur were: 0-0, 0-20, 0-40, 100-10, 100-30, 200-0, 200-20, 200-40, 300-10, 300-30, 400-0, 400-20 and 400-40 mg dm^{-3} , set in randomized block design, with four replications. Nitrogen and sulfur were applied as ammonium nitrate and calcium sulfate respectively, and the amount of calcium was balanced by using calcium chloride. For each grass growth period, sulfur rates were applied once and nitrogen rates were split into four applications, with interval of two days between them.

The fertilizations with other macronutrients were: phosphorus = 200 mg dm^{-3} ; potassium = 150 mg dm^{-3} and magnesium = 50 mg dm^{-3} , which were supplied after nitrogen applications with intervals of two days between applications, by using: $\text{Ca}(\text{H}_2\text{PO}_4)_2$, KH_2PO_4 , KCl and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

The additional supply of magnesium was 20 mg dm^{-3} due to non-application of phosphorus after the first harvest. Boron, copper, zinc and molybdenum were supplied before grass planting with the following sources and amounts: $\text{H}_3\text{BO}_3 = 1.5 \text{ mg dm}^{-3}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O} = 2.5 \text{ mg dm}^{-3}$, $\text{ZnCl}_2 = 2.0 \text{ mg dm}^{-3}$ and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O} = 0.25 \text{ mg dm}^{-3}$.

Irrigation procedure

Soil moisture was maintained by a subsurface self-irrigated system (Bonfim-Silva et al., 2007), which allowed the continuous water replacement according to evapotranspiration of the soil-plant system, ensuring the soil water retention capacity.

Plant management

Five seedlings of Marandu palisadegrass were cultivated per pot. Plants were harvested three times in the following growth periods: 35 days after the transplanting of seedlings, 28 days after the first harvest and 27 days after the second harvest. The plants were harvested 5 cm above the soil surface and all material collected was dried in a forced air ventilated oven at 65°C until constant mass was achieved and subsequently weighed.

At the harvesting time, the number of tillers, the number expanded leaves and leaf area of plants were also quantified in each pot. Leaf area was determined by using a leaf area machine model LI 3100 (LICOR®, NE, USA). Concentrations of total nitrogen and sulfur in shoot were determined according to Nelson and Sommers (1973) and Tabatabai (1982), respectively. The content of these nutrients in shoot dry matter were calculated.

Statistical analysis

The results were analyzed by using the Statistical Analysis System (SAS, 2008). At first, analysis of variance was performed for the N and S rates combinations. When the result was significant for the test F for nitrogen rates \times sulfur rates interaction, polynomial regression was applied using response surface regression (PROC RSREG). When that interaction was not significant, regression analysis for the components of the first and second degree was performed by using the PROC GLM (Montgomery, 2005). Significance level of 5% was used for all statistical tests.

Conclusion

During the establishment of Marandu palisadegrass, nitrogen supply, but not sulfur supply, is responsible for the increase in the number of tillers, number of leaves, leaf area, dry matter production and nitrogen content in the grass shoots. The lack of response to sulfur is due to the low demand for it in the initial development of this forage grass. To increase these plant attributes in the plant regrowth periods, nitrogen supply should be combined with sulfur fertilization. These results suggest that application of simultaneous sulfur and nitrogen fertilizer can decrease the amount of nitrogen required for high-yield grass production and reduce production costs.

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