

Diagnosing nitrogen and sulfur status in marandu palisadegrass via non-destructive and destructive sampling methods

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Abstract

The need for reliable strategies of nitrogen and sulfur fertilization has spurred a search for tools to diagnose levels of these nutrients in forage plants. The objective of this study was to diagnose nitrogen and sulfur status in *Brachiaria brizantha* cv. Marandu via destructive and non-destructive sampling methods by growing this forage grass with different combinations of nitrogen and sulfur in a Quartzipsamment soil with low organic matter content. The experiment was carried out in a greenhouse during the austral summer (December to April). The experiment had a 5² fractional factorial design with 13 combinations of nitrogen-sulfur rates, as follows: 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. Experimental units were distributed in a randomized block design with four replicates. Three growth periods were studied and diagnostic leaves (lamina of recently expanded +1 and +2 leaves) were harvested at the end of each period. The results showed that diagnostic leaves of nitrogen-deficient marandu palisadegrass plants had SPAD values of 16 to 20 and nitrogen concentrations of 11 to 14 g kg⁻¹. Nitrogen-sufficient grasses had a SPAD value of ≥ 40 -45 and nitrogen concentrations of >29.2 g kg⁻¹. The sulfur status of grasses depended on their supply of nitrogen. Diagnostic leaves of plants treated with nitrogen but not sulfur had SPAD values of 17-28, sulfur concentrations of 0.6-1.2 g kg⁻¹, sulfur-sulfate concentrations of 56-84 mg kg⁻¹, and N:S ratios of up to 80:1.

Keywords: *Brachiaria brizantha*; nutrient concentration; N:S ratio; SPAD values; sulfur-sulfate.

Abbreviations: SPAD values estimates of chlorophyll content; N_nitrogen; S_sulfur; N:S ratio_ nitrogen:sulfur ratio; P_phosphorus; K_potassium; Ca_calcium; Mg_magnesium; Al_aluminum; CEC_cation exchange capacity; SB_sum of bases; H+Al_potential acidity; V_base saturation; N-total_total nitrogen; N-NO₃⁻_nitrate nitrogen; N-NH₄⁺_ammonium nitrogen; S-SO₄⁻²_sulfate sulfur; SAS_Statistical Analysis System; F-tests_statistical test; RSREG_procedures_regression procedures; GLM_procedure_general linear model procedure; ANOVA_analysis of variance; PROC CORR_correlation procedures; R²_coefficient of determination.

Introduction

Nitrogen and sulfur are frequently studied in plant nutrition for two primary reasons: a) they occur in anionic form in soils (NO₃⁻ and SO₄²⁻) and move easily through the soil profile, and b) large quantities of the nitrogen and sulfur in soils occur in organic forms that are not immediately available to plants. Given their similarities, management practices that affect concentrations of one of these nutrients can also potentially alter those of the other (McNeill et al., 2005). When nitrogen levels in plant tissues are low, plants do not metabolize nutrients efficiently and do not make efficient use of sulfur (Conley et al., 2002). Conversely, sulfur-deficient crops cannot take full advantage of the benefits nitrogen offers for yield, quality, or protein content, and cannot make efficient use of nitrogen fertilizer (Sahota, 2006). In addition, sulfate acquired from the soil solution is carried to the symplast of the root cortex, from which it must be transported to the xylem apoplast, in order to be transported via leaf transpiration flux (Cram, 1983; Herschbach et al., 1995). In the leaves, sulfate is stored in the vacuole or metabolized in the reductive assimilation of sulfur, using the energy of photosynthesis (Kataoka et al., 2004). Due to the importance of both nitrogen and sulfur in protein composition, plant requirements of those nutrients are linked

(Jamal et al., 2010). It is understood that for every 15 parts of nitrogen in a protein one part of sulfur is needed, which implies a general N:S ratio of 15:1 in plants (Jamal et al., 2010). Sulfur-deficient plants may thus use less of the nitrogen in organic and mineral fertilizers, increasing the potential for nitrogen leaching (Schnug, 1997). Interest in establishing reliable strategies for nitrogen and sulfur fertilization has spurred a search for tools to diagnose levels of these nutrients in the leaves of forage plants (Mathot et al., 2009). Among the tools available for foliar diagnosis, SPAD values have emerged as one of the most rapid and inexpensive methods (Fox et al., 1994). SPAD values are determined with a portable chlorophyll meter capable of quick, non-destructive readings (Fahurrozi and Atewart, 2001). The effectiveness of SPAD chlorophyll meters in predicting nitrogen nutritional status is increasingly well documented, but it remains necessary to determine the range of SPAD values that reflect an adequate supply of nitrogen and sulfur (Swiader and Moore, 2002). Such data are especially scarce for tropical forage grasses. Since nitrogen is the main nutrient for forage grass productivity, and since grasses are fertilized with nitrogen, sulfur is usually the second most important nutrient for increasing pasture

production. However, destructive and non-destructive methods for diagnosing N and S status in diagnostic leaves of tropical forage grasses have not been compared. Non-destructive methods in particular have the potential to allow users to quickly determine pasture fertilization levels in the field. In this context, the objective of this study was to carry out nitrogen and sulfur diagnoses, via non-destructive and destructive sampling methods, of the tropical grass *Brachiaria brizantha* cv. Marandu, fertilized with nitrogen and sulfur in a pastureland soil with low levels of organic matter.

Results and discussion

Studies to date of plant nutritional status have mostly focused on nutrient concentrations in plant tissues. Where sulfur is concerned, useful variables include N:S ratios, sulfur-sulfate concentrations, and estimates of chlorophyll content (SPAD values) obtained via non-destructive sampling methods.

SPAD of diagnostic leaves

SPAD values of recently expanded leaves measured during the first growth period showed no significant nitrogen \times sulfur interaction, and no significant relationship with sulfur concentrations alone (Fig. 1a). However, SPAD values measured during the second and third growth periods showed a significant nitrogen \times sulfur interaction, with the results fitting a polynomial regression model (Figures 1b and 1c). Although the soil was poor in organic matter, SPAD values may not have responded to sulfur supply in the first growth period because the sulfur content was adequate for the grass given its nitrogen supply. SPAD value responses to nitrogen in the first growth period may be related to high photosynthetic activity (Haboudane et al., 2002). SPAD values of diagnostic leaves in the first growth period varied from 17.8 to 50.6, when comparing no added nitrogen with the nitrogen rate corresponding to the maximum SPAD value. According to Haboudane et al. (2002), the higher SPAD value, the greater the chlorophyll content and nitrogen concentration of the leaves. The maximum SPAD value in recently expanded leaves in the second growth period of marandu palisadegrass would be observed at a rate of nitrogen higher than the maximum used in this experiment, combined with a sulfur supply of 39.8 mg dm⁻³ (Fig. 1b). The response surface shows a plateau that begins with combinations of nitrogen concentrations near 350 mg dm⁻³ and sulfur concentrations near 35 mg dm⁻³ (a 10:1 ratio). In an experiment carried out with nutrient solution and an inert substrate, Batista and Monteiro (2007) observed in the second growth period of marandu palisadegrass a significant nitrogen \times sulfur interaction and higher SPAD values in recently expanded leaves, due to the increase in sulfur associated with the increase in nitrogen. Colozza et al. (2000) found maximum SPAD values of 32.7 and 39.7 in laminas of recently expanded leaves of aruana guineagrass (*Panicum maximum* cv. Aruana) in the first and second growth periods, respectively. They observed a maximum SPAD value of 38 for mombaça guineagrass. (*Panicum maximum* cv. Mombaça), in both the first and second growth periods. The highest SPAD value (44.6) in the laminas of recently expanded leaves in the third growth period were seen at a nitrogen concentration of 377.2 mg dm⁻³ and a sulfur concentration of 35.4 mg dm⁻³, a ratio of 10.7:1 (Fig. 1c). In marandu palisadegrass plants not treated with nitrogen, SPAD values of 17.8, 16.7, and 20.5 (Figures 1a, 1b, and 1c) were observed in the first, second, and third growth periods,

respectively. These values indicate severe nitrogen deficiency for the grass. SPAD values in the first growth period (when changes in SPAD values were only observed with variation in nitrogen) were significantly and positively correlated with the nitrogen concentrations of diagnostic leaves at all concentrations of nitrogen supply ($r=0.63^{**}$). This confirms that SPAD values can be used to assess the nutritional status of grasses with regard to nitrogen, as other authors have argued (Colozza et al., 2000; Batista and Monteiro, 2007). Figures 1a and 1b show that in the absence of sulfur, SPAD values increase with increasing nitrogen concentrations up to 200 mg dm⁻³. However, the highest SPAD values were obtained under a combination of high nitrogen and sulfur concentrations. These changes are the result of changes in the chlorophyll concentration of the grasses, which alter the intensity of the green color with varying ratios of nitrogen to sulfur (Schonhof et al., 2007). According to Bushuk et al. (1978), an increase in photosynthetic rate is also expected in plants grown with higher levels of sulfur. In samples collected at the second harvest, plants grown without sulfur showed SPAD values that increased from 17 to 24 as nitrogen supply increased from 50 to 250 mg dm⁻³ (Figure 1b). In samples collected at the third harvest, SPAD values increased from 20 to 28 as nitrogen supply increased from 50 to 200 mg dm⁻³ (Fig. 1c). This range of SPAD values (17-20) indicates sulfur deficiency in the leaves. The highest SPAD values were observed in samples collected at the second and third harvests from plants grown with 400 mg dm⁻³ nitrogen and 40 mg dm⁻³ sulfur (a 10:1 ratio), i.e., an adequate supply of both nutrients.

Nitrogen in diagnostic leaves

There was no significant nitrogen \times sulfur interaction for the concentrations of nitrogen in the diagnostic leaves collected at the first and second harvests. There was a significant correlation, however, between diagnostic leaf nitrogen content and nitrogen levels in the nutrient solution (Figures 2a and 2b). These results are in agreement with those of Mathot et al. (2008), who found no effect of sulfur supply on nitrogen concentrations of the forage grasses *Lolium perenne* and *Lolium multiflorum*. Nitrogen concentrations in the diagnostic leaves collected at the first and second harvests showed a linear correlation (Figures 2a and 2b) with nitrogen levels in the nutrient solution. The highest nitrogen concentrations in diagnostic leaves were 33.3 g kg⁻¹ in the first harvest and 28.0 g kg⁻¹ in the second harvest, both observed at the highest nitrogen supply (400 mg dm⁻³). These maxima (indicative of adequate nitrogen nutritional status) were 2.9 and 2.1 times higher than those observed in plants not treated with nitrogen (11.5 g kg⁻¹ and 13.0 g kg⁻¹), respectively. These nitrogen concentrations (11.5 g kg⁻¹ and 13.0 g kg⁻¹) in the diagnostic leaves of marandu palisadegrass indicate nitrogen deficiency, probably due to the low organic matter content, which resulted in low nitrogen mineralization. At the first harvest, the maximum soil organic matter content (15.6 mg dm⁻³) was observed at a nitrogen supply level of 207.1 mg dm⁻³, and mean total nitrogen content in soils was 0.5 g kg⁻¹. The highest soil nitrate content was 12 mg kg⁻¹, observed at a nitrogen supply of 112.7 mg kg⁻¹, and mean soil ammonium values varied from 13.9 to 24 mg kg⁻¹. However, maximum soil organic matter content at the second harvest (12.8 mg dm⁻³) was 1.2 times lower than at the first harvest, even after receiving a higher nitrogen supply (245.3 mg dm⁻³). In that case, the mean total nitrogen soil content was 0.4 g kg⁻¹, while nitrate and ammonium soil contents varied from 22.3 to 166.4 mg kg⁻¹ and from 82.4 to

111.9 mg kg⁻¹ in plants that received no nitrogen and those that received 400 mg dm⁻³, respectively. These results in the diagnostic leaves collected at the first and second harvests corroborate Whitehead's (1995) observation that when nitrogen fertilizer levels are very low for grasses that are severely deficient in nitrogen, the nitrogen concentrations of diagnostic leaves change little or not at all. When such plants receive high levels of nitrogen, however, nitrogen concentrations in diagnostic leaves show a marked increase. The nitrogen concentration of diagnostic leaves collected at the third harvest showed a significant nitrogen × sulfur interaction, and the data fit a polynomial regression model (Figure 2c). At low levels of nitrogen supply (0-100 mg dm⁻³), nitrogen concentrations in diagnostic leaves declined with increasing sulfur supply. However, at high levels of both nitrogen and sulfur supply, nitrogen concentrations in diagnostic leaves were high. These results indicate that high levels of nitrogen fertilizer should be accompanied by compatible amounts of sulfur (Flaete et al., 2005). Changes in the nitrogen concentrations of diagnostic leaves collected at the third harvest are in agreement with Gierus et al. (2005) recommendation that nitrogen fertilization at a rate of 300 kg ha⁻¹ should be accompanied by sulfur fertilization at a rate of 25 kg ha⁻¹, and that nitrogen fertilization of less than 300 kg ha⁻¹ does not require accompanying sulfur fertilization. Nitrogen concentrations in the diagnostic leaves collected at the third harvest varied from 14.4 to 38.3 g kg⁻¹ (Fig. 2c). Based on those values and the total aboveground dry mass production, the critical level of nitrogen in diagnostic leaves was calculated for marandu palisadegrass. For diagnostic leaves collected at the second and third harvests, these critical level values were 29.2 g kg⁻¹ ($Y = -32.3920 + 3.6373N - 0.06239N^2$) and 29.7 g kg⁻¹ ($Y = -8.8073 + 2.0230N - 0.03401N^2$), respectively. As seen in leaves collected at earlier sampling periods, the concentration of 14.4 g kg⁻¹ in the diagnostic leaves indicated nitrogen deficiency in the marandu palisadegrass; mean values of soil organic matter, total nitrogen, and ammonium were 8.37 mg dm⁻³, 0.3 g kg⁻¹, and 18.8 mg dm⁻³, respectively; and the lowest nitrate concentration (19.6 mg kg⁻¹) was associated with a supply of 131.9 mg dm⁻³.

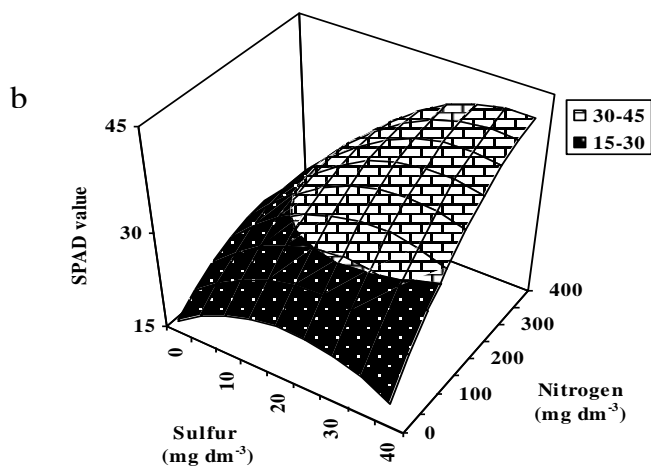
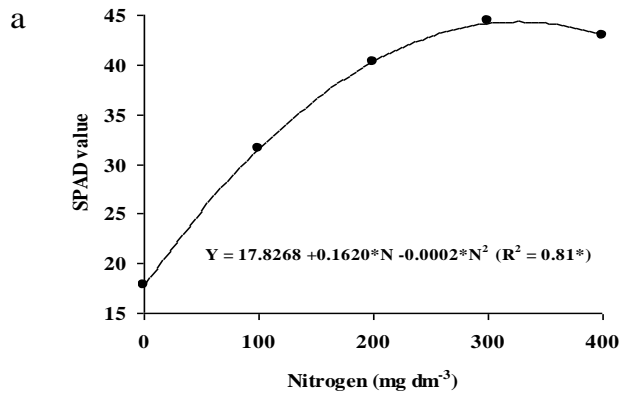
Sulfur in the diagnostic leaves

There was a significant nitrogen × sulfur interaction for sulfur concentrations in diagnostic leaves collected at all three harvests (Fig. 3). These results may be related to the slow release rate of sulfur by soil organic matter (Saeed et al., 2012) associated with the low organic matter contents observed at the first, second, and third harvests: 15.6 mg dm⁻³, 12.8 mg dm⁻³, and 8.37 mg dm⁻³, respectively. For diagnostic leaves collected at the first harvest, a saddle point was observed in the response surface and neither maxima nor minima could be determined. However, the highest concentrations of sulfur in diagnostic leaves (1.5 to 1.8 g kg⁻¹) were observed in plants treated with a combination of high supply rates of nitrogen (350 to 400 mg dm⁻³) and intermediate to high supply rates of sulfur (20 to 40 mg dm⁻³; Figure 3a). At the first harvest, mean total sulfur soil content was 105.73 mg kg⁻¹, while sulfate content showed a positive linear correlation with sulfur supply, varying from 11.4 to 27.6 mg kg⁻¹. The combination of 0-150 mg dm⁻³ nitrogen supply and 0-30 mg dm⁻³ sulfur supply yielded increased sulfur concentrations in diagnostic leaves collected at the first harvest, with values varying from 0.6 to 1.2 g kg⁻¹ (Fig. 3a). However, at a sulfur supply of ≥35 mg dm⁻³, increases in sulfur yielded lower sulfur concentrations in the diagnostic

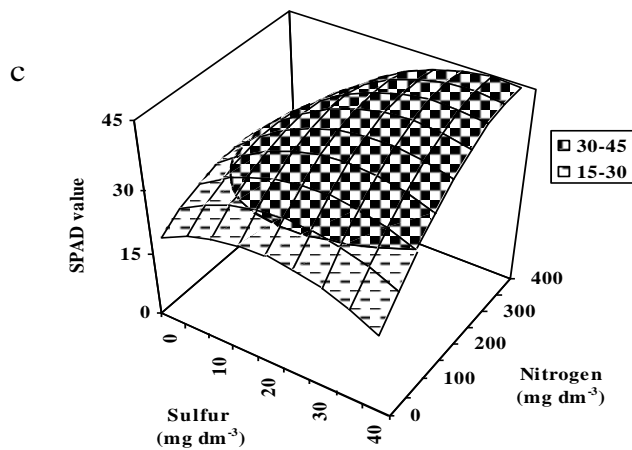
leaves (1.2 a 0.7 g kg⁻¹), indicating a dilution effect due to the increased dry mass production caused by sulfur application. In the leaves collected at the second and third harvests, the saddle point in the response surface analysis did not allow us to determine the nitrogen-sulfur combination that yielded the largest or smallest sulfur concentrations in diagnostic leaves. However, in the leaves collected at the second harvest, for a given supply level of nitrogen (0 to 400 mg dm⁻³), an increase in the sulfur supply up to 20 mg dm⁻³ increased sulfur concentrations in leaves, with values varying from 0.7 to 1.2 g kg⁻¹. At sulfur supply levels from 25 to 35 mg dm⁻³ leaf sulfur concentrations remained constant, with values of 1.2 and 1.3 g kg⁻¹ (Fig. 3b). A sulfur concentration of ≥1.2 g kg⁻¹ in diagnostic leaves collected at the second harvest thus indicated adequate nutrition. As seen in the leaves collected at the first harvest, for a given supply level of sulfur leaves collected at the second harvest also showed increasing sulfur concentrations in diagnostic leaves with increasing nitrogen supply, up to 150 mg dm⁻³. Above that concentration, additional nitrogen caused a reduction in leaf sulfur. At combinations of nitrogen levels of 100 to 250 mg dm⁻³ and sulfur levels of 25 to 30 mg dm⁻³ a plateau was observed (Fig. 3b), which makes evident that sulfur deficiency depended on the rate this nutrient was applied to grow this grass (Jamal et al., 2010). In the diagnostic leaves collected at the third harvest, at nitrogen supply levels of 0-50 mg dm⁻³, increasing supply of sulfur above 15 mg dm⁻³ yielded higher sulfur concentrations in diagnostic leaves (Fig. 3c). These results once again highlight the fact that increased nitrogen supply is inefficient when the sulfur supply is insufficient to satisfy plant requirements. Results from the first, second, and third harvests showed that the efficient use of sulfur is often connected to both the amount available to plants and the requirements for dry mass production, which depends on the quantity and quality of its supply (McNeill et al., 2005). It is also important to note that in addition to the low soil organic matter content, the mean total sulfur soil content at the second harvest was 102.68 mg kg⁻¹, while sulfate content showed a linear response. As nitrogen supply increased, sulfate content decreased (55 to 30.8 mg dm⁻³), and as sulfur supply increased, sulfate content increased (12.2 to 75.2 mg dm⁻³). At the third harvest, increasing supply levels of sulfur yielded a linear response in total sulfur content (88.4 to 131.8 mg kg⁻¹) and in sulfate sulfur (18.8 to 81 mg dm⁻³). The critical level of sulfur in the diagnostic leaves was calculated based on the sulfur concentrations in the diagnostic leaves and the total aboveground dry mass production. The critical level of sulfur was 1.11 g kg⁻¹ ($Y = 7.2699 + 13.0034S - 5.8513S^2$) for diagnostic leaves collected at the second harvest, and 0.79 g kg⁻¹ ($Y = -8.7494 + 16.3988S - 10.2801S^2$) for leaves collected at the third harvest

N:S ratio in the diagnostic leaves

There was no significant nitrogen × sulfur interaction for the N:S ratio of diagnostic leaves collected at the first harvest, and the ratio was also not correlated with nitrogen or sulfur supply alone. There was a significant nitrogen × sulfur interaction for the N:S ratio in leaves collected in the second and third harvests. In diagnostic leaves collected at the second harvest, the N:S ratio fit a polynomial regression model, but due to the saddle point in the response surface it was not possible to determine the maxima or minima of the relationship. In plants supplied with high levels of nitrogen (400 mg dm⁻³) but no sulfur, very high N:S ratios (approximately 80:1) were observed in the diagnostic leaves (Figure 4a), and these values were associated with very low sulfur concentrations (0.4 g kg⁻¹).

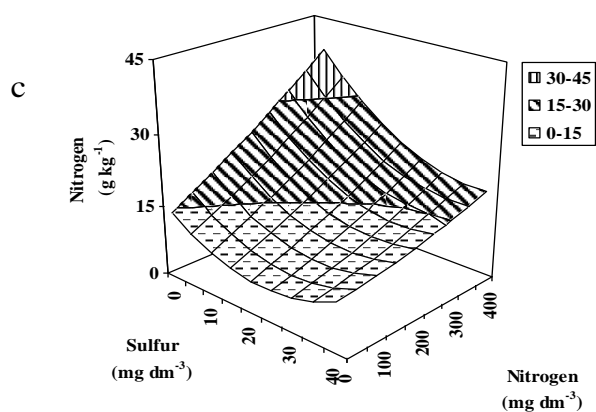
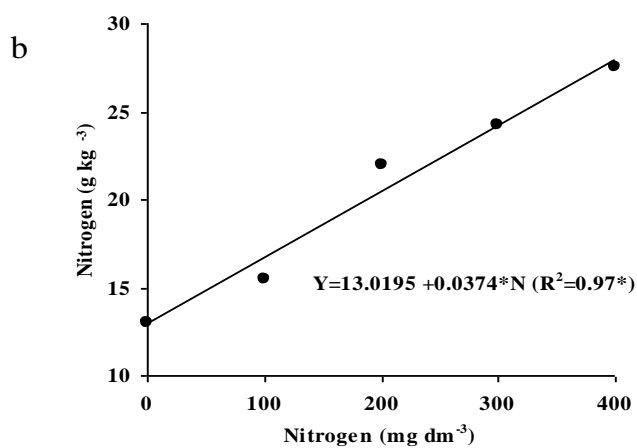
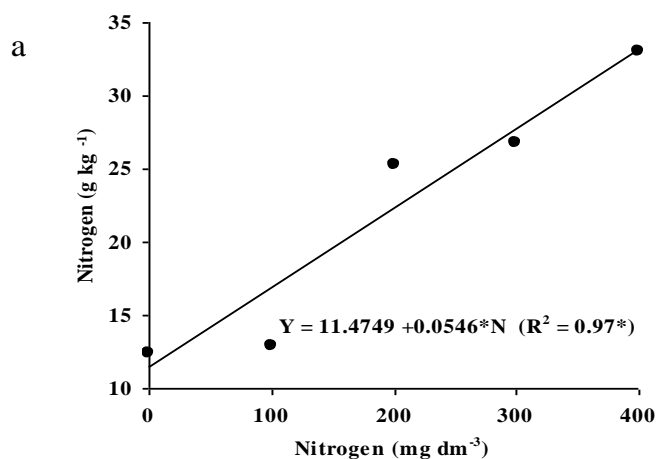


$Y = 16.7056 + 0.0550*N - 0.0001*N^2 + 0.6130*S - 0.0139*S^2 + 0.0011*NS$ ($R^2 = 0.82*$)



$Y = 20.4772 + 0.0686*N - 0.0001*N^2 + 0.6355*S - 0.0170*S^2 + 0.0015*NS$ ($R^2 = 0.71*$)

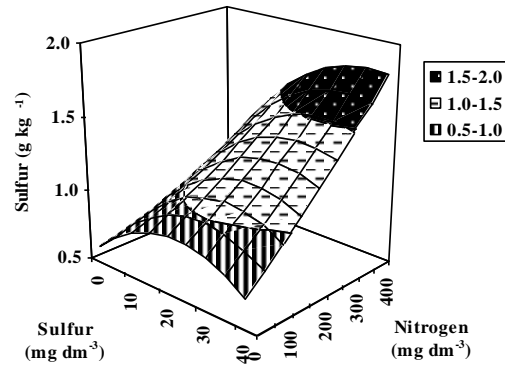
Fig 1. SPAD value in the diagnostic leaves of marandu palisadegrass sampled at the first (a), second (b) and third (c) harvests, as related to nitrogen rates (a) and nitrogen and sulfur combinations (b,c).



$$Y = 14.3598^* + 0.0495^*N + 0.00003^*N^2 - 0.6505^*S + 0.0138^*S^2 - 0.0010^*NS \quad (R^2 = 0.78^*)$$

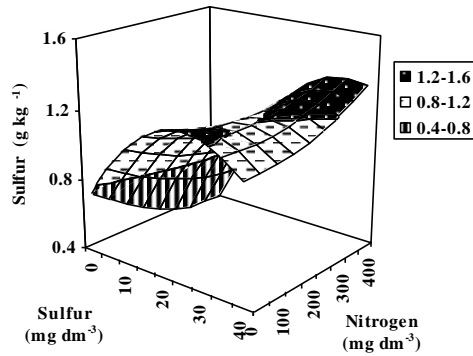
Fig 2. Nitrogen concentrations in the diagnostic leaves of marandu palisadegrass sampled at the first (a), second (b) and third (c) harvests, as related to nitrogen rates (a,b) and nitrogen and sulfur combinations (c).

a



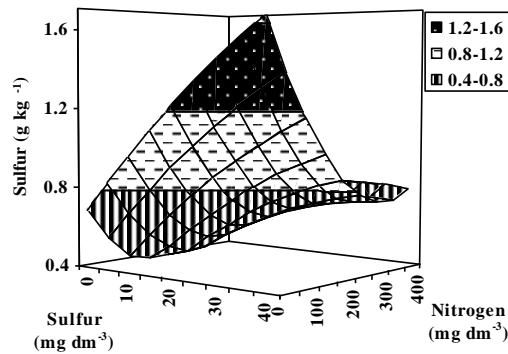
$$Y=0.6109 + 0.0006*N + 0.000001*N^2 + 0.0265*S - 0.0006*S^2 + 0.00004*NS \quad (R^2=0.72^*)$$

b



$$Y=0.7355 - 0.0016*N + 0.000002*N^2 + 0.0399*S - 0.0008*S^2 + 0.00004*NS \quad (R^2=0.56^*)$$

c

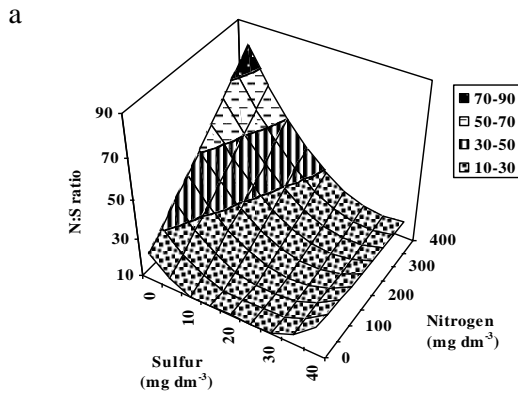


$$Y = 0.6890 - 0.0035*N + 0.000009*N^2 + 0.0304*S - 0.0002*S^2 - 0.00005*NS \quad (R^2=0.59^*)$$

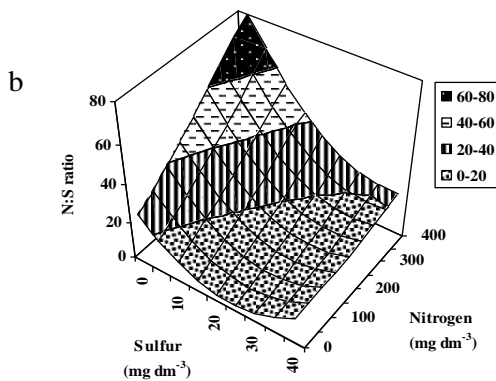
Fig 3. Sulfur concentrations in the diagnostic leaves of marandu palisadegrass sampled at the first (a), second (b) and third (c) harvests, as related to nitrogen and sulfur combinations.

High N:S ratios (such as 80:1) demonstrate severe sulfur deficiency in the grass. Dijkshoorn and van Wijk (1967) have argued that an N:S ratio of 13.7:1 is adequate for grasses, while other authors (Colozza et al., 2000; Batista and Monteiro, 2007; Mathot et al., 2008; Mathot et al., 2009; Jamal et al., 2010) have confirmed that N:S ratios of 10:1 to 16:1 reflect healthy nutritional levels for grasses. The high N:S ratios observed reinforce the fact that when the nitrogen fertilizer

supply to a pastureland is increased the sulfur supply should also be increased in order to guarantee a balance of the two nutrients for adequate plant nutrition (Malavolta, 2006). In the plant tissue collected at the second harvest, for a given nitrogen supply level, increasing sulfur concentrations up to 25 mg dm⁻³ yielded N:S ratios that were roughly four times lower (51.1 vs. 12.9). For a given sulfur supply level, increasing nitrogen concentrations yielded N:S ratios that were up to seven times



$$Y=24.2468 + 0.1284*N + 0.00003*N^2 - 1.9979*S + 0.0495*S^2 - 0.0038*NS \quad (R^2=0.85^*)$$



$$Y=26.709202 + 0.1155*N + 0.00006*N^2 - 2.0178*S + 0.0426*S^2 - 0.0030*NS \quad (R^2=0.74^*)$$

Fig 4. Nitrogen:Sulfur (N:S) ratio in the diagnostic leaves of marandu palisadegrass sampled at the second (a) and third (b) harvests, as related to nitrogen and sulfur combinations.

higher (4.1 vs. 29.4) (Fig. 4a). These results suggest that nitrogen-deficient marandu palisadegrass does not respond to sulfur fertilization. Figure 4a shows a plateau of low N:S ratios (approximately 4:1) between sulfur supply levels of 15 to 25 mg dm⁻³ in plants not treated with nitrogen. The N:S ratio varied from 4.3:1 to 80.3:1. Batista and Monteiro (2007) reported that N:S ratios in the diagnostic leaves of marandu palisadegrass collected at the second harvest varied from 2.8:1 to 37.2:1. N:S ratios in diagnostic leaves of marandu palisadegrass collected at the third harvest fit a polynomial regression model that included nitrogen and sulfur supply (Fig. 4b). For a given supply level of nitrogen, the N:S ratio in diagnostic leaves collected at the third harvest remained stable with increasing sulfur supply (Fig. 4b). However, for a given supply level of sulfur, the N:S ratio increased with increasing nitrogen supply, which suggests possible sulfur deficiency, as reported by Mathot et al. (2008). The proper combination of nitrogen and sulfur supply has an important effect on the absorption and concentration of these nutrients in grasses (Jamal et al., 2010). The highest N:S ratios (approximately 82:1) in diagnostic leaves of marandu palisadegrass collected at the third harvest were observed in plants treated with a high level of nitrogen (400 mg dm⁻³), regardless of the sulfur supply, while the lowest values (3:1) were observed in plants that received no nitrogen and sulfur concentrations of 20 to 25 mg dm⁻³ (Fig. 4b). The 82:1 N:S

ratio in the diagnostic leaves indicates sulfur deficiency in the grass and was observed at a sulfur concentration of 0.7 g kg⁻¹. The results of this analysis of marandu palisadegrass nutrition illustrate the importance of maintaining an adequate N:S ratio in order to synchronize nitrogen and sulfur management strategies to satisfy plant requirements (McNeill et al., 2005).

Sulfur-sulfate in the diagnostic leaves

There was no significant nitrogen × sulfur interaction for sulfur-sulfate concentrations in recently expanded marandu palisadegrass leaves collected at the first and second harvests. A significant difference was observed between plants supplied with 0 and 20 mg dm⁻³ of sulfur for all treatments, except those collected at the second harvest (Figures 5a, 5b, and 5c). Marandu palisadegrass plants grown without sulfur showed the lowest values of sulfur-sulfate in the diagnostic leaves, varying from 83.6 to 55.8 mg kg⁻¹ in material collected at the three harvests. These results show that: a) the available sulfur in the soils (S-SO₄²⁻ = 7.3 mg dm⁻³) was sufficient to satisfy the requirements of the grass during the first growth period, and/or b) marandu palisadegrass requires more sulfur after the establishment stage. As a result, sulfur-sulfate concentrations decline in the grass during the regrowth stage (i.e., after harvests), and sulfur fertilization is necessary.

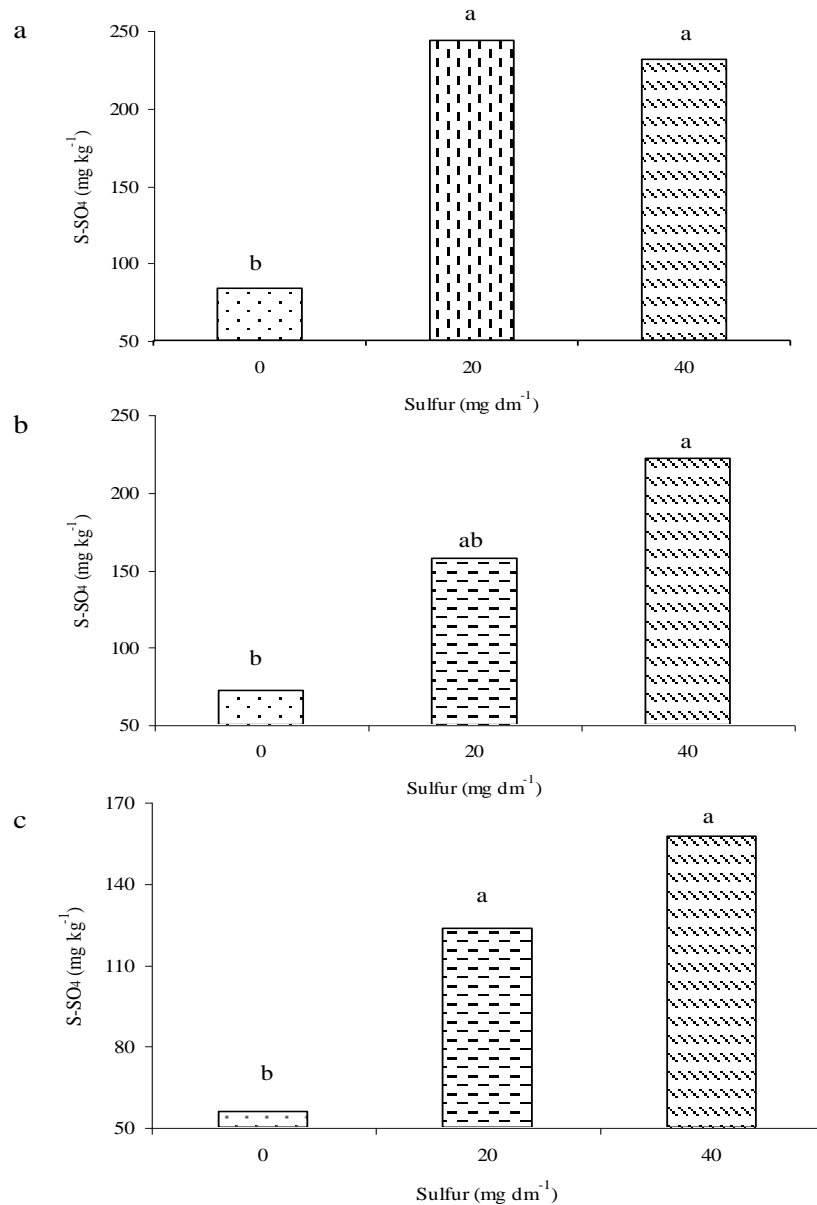


Fig 5. Sulfate-sulfur (S-SO₄) concentrations in the diagnostic leaves of marandu palisadegrass sampled at the first (a), second (b) and third (c) harvests, as related to sulfur rates. Means in each sub-figure followed by different letters are significantly different by the Tukey test at $p \leq 0.05$.

Materials and methods

Growth conditions, plant species, and soils

The experiment was carried out in a greenhouse in Piracicaba, São Paulo state, Brazil, using the forage grass *Brachiaria brizantha* cultivar Marandu, in a Quartzipsamment soil during the austral summer (December 2004 to April 2005). Samples of soils with low organic matter content were collected on a farm in Piracicaba, from a degraded pasture of signal grass (*Brachiaria decumbens*). Soils were collected at a depth of 0-20 cm, air dried in the shade, sieved, and placed in 3.6-dm³ plastic pots. Initial analyses of the soils used in the experiment yielded the following results: pH in CaCl₂=4.08; organic matter content = 11.6 g dm⁻³; P=5.34 mg dm⁻³; K=1.75 mmol_c dm⁻³; Ca=4.0 mmol_c dm⁻³; Mg=2.3 mmol_c dm⁻³; Al=4 mmol_c dm⁻³; H +

Al=21.5 mmol_c dm⁻³; SB=8.0 mmol_c dm⁻³; CEC=29.5 mmol_c dm⁻³; V=26.8%; N-total=0.32 g kg⁻¹; N-NO₃⁻= 21.6 mg kg⁻¹; N-NH₄⁺= 24.5 mg kg⁻¹; and S-SO₄⁻²= 7.3 mg dm⁻³. Liming was deemed necessary, using a V₂ of 50% in the formula used to calculate the amount of lime, according to Werner et al. (1996). Liming equivalent to 6000 kg ha⁻¹ was carried out by applying calcium oxide and magnesium oxide, after which the soil was watered and incubated for 31 days.

Nutrient applications and plant harvests

Five rates of nitrogen (0, 100, 200, 300, and 400 mg dm⁻³) and five rates of sulfur (0, 10, 20, 30, and 40 mg dm⁻³) were combined in a 5² fractional factorial design based on Littell and Mott (1975). There were thus 13 nitrogen-sulfur combinations, as follows: 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. Nitrogen was supplied as ammonium nitrate and sulfur as calcium sulfate, both in solution. The experimental was set in randomized blocks with four replicates. After soils were incubated, nitrogen and sulfur were added together with other nutrients as described in Batista and Monteiro (2010). Marandu palisadegrass seedlings were obtained by germinating seeds in trays of rinsed sand and 15 seedlings were transplanted to each pot. One week after transplanting and nutrient application, seedlings were observed to have been burned, probably due to the relatively high osmotic pressure. After this was observed, plants were harvested and soils rinsed with deionized water for 33 days. The leached solutions were re-applied to their respective pots after new seedlings had been transplanted. New marandu palisadegrass seedlings were germinated and transplanted to the pots. After a period of adaptation, plants were thinned until five remained in each pot. Plants were harvested three times: once 38 days after the seedlings were transplanted, once 27 days after the first harvest, and once 38 days after the second harvest. After each harvest, a fourth of the total nitrogen rates was added every two days in order to avoid an abrupt change in the osmotic pressure of the root environment. Sulfur was added in a single application after the nitrogen treatment was complete. Magnesium chloride and calcium chloride were added to ensure equal amounts of calcium for all nitrogen-sulfur combinations, with an interval of two days between treatments. Micronutrients were added four days after the application of the calcium chloride.

SPAD readings

An SPAD-502 chlorophyll meter (Soil-Plant Analysis Development, Minolta Camera Co., Osaka, Japan) was used to estimate chlorophyll content. This equipment was managed following the instructions described in the user's manual. Non-destructive readings were taken directly from the middle third of the lamina of the second fully expanded leaf, taking care to avoid the midvein, on three occasions: 17 days after the last seedlings were thinned, 23 days after the first harvest, and 24 days after the second harvest. Five readings were taken per pot to yield mean values for each nitrogen-sulfur combination.

Nitrogen and sulfur determinations in diagnostic leaves

Laminas of recently expanded leaves [+1 and +2] with a visible ligule (diagnostic leaves) were collected at each harvest. Two diagnostic leaves were collected from each tiller. Leaf sheaths were not included in these samples. Harvested material was dried in a forced-air circulation oven at 70°C until it reached a constant mass, and later ground in a Wiley mill. Nitrogen and sulfur concentrations of the milled samples were determined following Sarruge and Haag (1974). Nitrogen was determined by the semi-micro Kjeldahl method after sulfuric digestion, while nitric-perchloric digestion was used to prepare the extracts in which sulfur concentrations were determined, via barium chloride turbidimetry following Sarruge and Haag (1974). Inorganic or free sulfate sulfur was determined by a procedure modified from Sinclair (1974). To determine the critical level, aboveground dry mass production (90% of maximum production) in each of the harvests was correlated with nitrogen and sulfur concentrations in the diagnostic leaves (Malavolta, 2006).

Statistical analysis

All data were analyzed statistically with SAS software using a 5% significance level. Data from nitrogen-sulfur combinations were compared using analysis of variance. When F-tests showed a significant nitrogen × sulfur interaction, data were subjected to polynomial regression (response surface) using the RSREG procedure. When no significant interaction was detected, first- and second-degree regression was carried out using the GLM command for nutrient concentrations with a significant effect in the ANOVA. Means were compared with Tukey's test at a 5% significance level. PROC CORR was used to correlate data related to nitrogen concentration, sulfur concentration, and SPAD value.

Conclusions

Nitrogen-deficient marandu palisadegrass plants showed SPAD values of 16 to 20 and nitrogen concentrations of 11 to 14 g kg⁻¹ in diagnostic leaves. Nitrogen-sufficient plants showed SPAD values of ≥40-45 and nitrogen concentrations of >29.2 g kg⁻¹. Sulfur nutritional status in this forage grass depends on nitrogen supply. Plants with sufficient nitrogen but insufficient sulfur showed SPAD values in the diagnostic leaves of 17-28, sulfur concentrations of 0.6-1.2 g kg⁻¹, sulfur-sulfate concentrations of 56-84 mg kg⁻¹, and N:S ratios as high as 80:1.

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