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ABSTRACT

The deficiency of sulfur (S) in agricultural soils has been aggravated in recent decades, mainly by the application of high concentration fertilizers that are S-free and intensive agricultural activities. This study evaluated forage yield, nutritional status and inorganic sulfate-S in soil when submitted to levels and sources of sulfur, including elemental sulfur, of the 'Mombaça' grass in a Typic Ultisol. The experimental design was completely randomized arrangement in a 5 × 4 factorial design, with five S rates (0, 50, 100, 150, and 200 mg kg^{-1}) and four S sources. The sources were elemental sulfur pastilles (ESPA), phosphogypsum (GY), gypsite (GI), and elemental sulfur powder (ESPO). The results showed that the S-use efficiency with the GI, GY and ESPO sources was obtained with the supply of 50 mg kg^{-1} of S, and was enough to optimize the 'Mombaça' grass yield and promote a better quality for the nutritional status of the forage. The ESPA source had an S leaf concentration below the adequate range and resulted in lower yield values in relation to the other S sources evaluated. The SPAD index in the newly expanded leaves was shown to be an indicator of shoot dry weight (SDW) yield and could be used as a pre-diagnosis of the S-efficiency in 'Mombaça' grass. The soil processes associated to the availability of S had a great influence on the granulometry, which drove the SDW yield and S use efficiency with the source ESPO in relation to ESPA.

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KEYWORDS
Fertilizer application; soil fertility; sulfate; pasture; *Megathyrsus maximus*

Introduction

Pastures comprise about 26% of the world's total land area and 80% of agricultural land (Boval and Dixon 2012; Wright et al. 2006). They are used mainly as a food source for meat and milk production, especially in tropical regions with high temperatures and humidity that favor the growth of C4 forage plants throughout the year (Moreno et al. 2014).

'Mombaça' grass (*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs) stands out because of its large size, high forage yield capacity, leaf blades and vigorous sprouting after defoliation (Canto et al. 2012; Heinrichs et al. 2016; Jank et al. 2008). However, tropical soils are acidic and highly weathered, resulting in low nutrients levels, especially sulfur (S), which is essential for forage yield (Fonte et al. 2014; Nesper et al. 2015). In addition, soil S scarcity has been aggravated in the last decades due to the excessive use of S-free high-concentration fertilizers and the intensification of agricultural production (Ryant and Skládanka 2009; Salvagiotti et al. 2012).

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The S supply is preponderant in the nutrition and recovery of forage plants in performing catalytic, regulatory and structural functions. This element is part of iron-sulfur (Fe-S) heme proteins, which exert key biological functions, such as photosynthesis, cellular respiration, nitrogen (N) and S metabolism, and hormone synthesis and gene expression regulation (Balk and Pilon 2011; Capaldi et al. 2015; Ding et al. 2016).

The S inorganic supply by means of fertilizers generally occurs as sulfate (SO_4^{2-}) or elemental S (S^0). Sulfate is readily available to be absorbed by plants, but is highly mobile in the soil and susceptible to leaching. The S^0 is a more concentrated form with the benefit of low transport costs and reduced leaching, but it becomes available only after oxidation (Degryse et al. 2016). This oxidation process can be affected by temperature, humidity, and pH conditions and soil organic matter (SOM) content (Brahim et al. 2017; Degryse et al. 2018).

The highest concentration of S^0 in fertilizer is found in the form of granules or pastilles, usually made up of 80% or more of S^0 and the remainder of a small amount of binder, commonly being bentonite, in addition of macronutrients enriched with S^0. The S^0 fertilizer is generally not used, since it presents a risk of explosion and difficulties in the handling of the powder (Chien, Gearhart, and Villagarcía 2011; Degryse et al. 2016).

The choice of granulometry of S^0 fertilizers can directly influence the efficacy, since the increase of the particle size decreases the S^0 oxidation, and is able to reduce the losses by leaching in the SO_4^{2-} form substantially and, at the same time, be favorable for a balanced and continuous supply during a crop's cycle (Germida and Janzen 1993; Janzen and Karamanos 1991). On the other hand, slow oxidation could cause S deficiency when the requirement of this nutrient is not supplied to the crop (Janzen and Bettany 1987).

Several studies on the nutritional status of forage plants have reported the importance of S supply in relation to the increased yield (Custódio et al. 2005; Lavres Júnior, Monteiro, and Schiavuzzo 2008; Moreira, Carvalho, and Evangelista 1997; Moreira, Evangelista, and Carvalho 1998; Schmidt and Monteiro 2015). However, there is little information on the most effective way to provide S for the development and quality of the 'Mombaça' forage grass.

This study evaluated forage yield, nutritional status and inorganic sulfate-S in soil submitted to S rates and sources in 'Mombaça' grass (*Megathyrsus maximus*) cultivated in a Typic Ultisol.

Materials and methods

Local place and experimental condition

The experiment was conducted under greenhouse conditions at São Paulo State University (UNESP), Dracena County, São Paulo State (21°29′ LS and 51°2′ LW at a 396 m altitude), Brazil. The soil was a Typic Ultisol (Santos et al. 2018) and the sample was collected at a 0–0.2 m depth, forming a composite sample. The soil was homogenized, air-dried and sieved with a 4 mm mesh. The soil chemical attributes were: pH ($CaCl_2$) = 4.6, soil organic matter (SOM) = 17.0 g kg^{-1}, available P (resin) = 6.0 mg kg^{-1}, poatassium (K^+) = 1.2 $mmol_c$ kg^{-1}, calcium (Ca^{2+}) = 6.0 $mmol_c$ kg^{-1}, magnesium (Mg^{2+}) = 3.0 mmol kg^{-1}, sulfate-sulfur ($S\text{-}SO_4^{2-}$) = 7.0 mg kg^{-1}, potential acidity (H+ Al) = 21.0 $mmol_c$ kg^{-1}, aluminum (Al^{3+}) = 3.0 $mmol_c$ kg^{-1}, sum of bases (SB) = 10.2 $mmol_c$ kg^{-1}, cation exchange capacity (CEC) = 31.2 $mmol_c$ kg^{-1}, and base saturation (V) = 32.7%.

For the soil acidity correction, the base saturation of soil was increased to 70% by adding calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) p.a. reagents at ratio of 3:1 (v:v). The soil with carbonate was incubated for 30 days in the pots awaiting reaction, while maintaining moisture at 80% field capacity.

At the end of the incubation period, the soil was air-dried for seven days. The soil of each pot (4.0 kg^{-1}) was filled in plastic trays where the treatments and fertilizers were applied according to the described by Santos et al. (2019) (mg kg^{-1}): 300 of N [urea $CO(NH_2)_2$, 45% of N], 200 of P [monocalcium phosphate $Ca(H_2PO_4)_2$], 150 of K [potassium chlorate (KCl, 60% of K_2O)], 0.5 of

B [boric acid (H_3BO_3, 18% of B)], 0.05 of Co [cobalt chloride ($CoCl_2$)], 1.0 of Cu [copper sulfate ($CuSO_4$, 13% of Cu)], 0.05 of Mo [molybdic acid (H_2MoO_4)], 0.05 of Ni [nickel sulfate ($NiSO_4$)], 5.0 of Mn [manganese sulfate ($MnSO_4 \cdot 5H_2O$), 26% of Mn] and 2.0 of Zn [zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 12% of Zn].

Experimental design

The experimental design was completely randomized arrangement in a 5 × 4 factorial design, with five S rates (0, 50, 100, 150, and 200 mg kg^{-1}), four S sources and four replicates. These sources were elemental sulfur pastilles (ESPA, 90% of S), with size of 1–4 mm; gypsum (GY) residue from the manufacture of phosphate fertilizers (24% of Ca and 17% of S); gypsite (GI), natural rock (24% of Ca and 17% of S) and elemental sulfur (S^0) powder (ESPO, 95% of S), with particles <0.1 mm.

Experimental management

The forage species used was *Megathyrsus maximus* 'Mombaça'. The study was carried out between October 2017 and January 2018, during which three cuts were made on 11/27/2017, 12/25/2017 and 01/22/2018. The 'Mombaça' grass was seeded in a tray with sand, where the seeds remained for 10 days and were then transplanted six seedlings per pot. After seven days the thinning was performed, leaving four uniform seedlings per pot. The irrigation of the experiment was carried out manually with deionized water, while maintaining the humidity at 80% of field capacity.

Growth and forage yield

The 'Mombaça' grass was cut with 7.0 cm above the ground surface. The first cut was 33 days after sowing, and the other two cuts were performed at 28 days intervals. In each cut, the morphological separation of leaves and stems+sheaths were performed. The material was dried at 65 ± 2°C to a constant weight (72 h) to determine the shoot dry weight (SDW), expressed in g per pot. The SDW was obtained from the leaves and stems + sheaths sum, while the leaves:stem ratio was the quotient between the dry weight of the leaf blades and the dry weight of stems+sheaths. Additionally, the tiller count was performed immediately prior to each cut an expressed in number per pot.

At the end of experiment, the roots of each pot were collected and washed on a 2.0 mm screen and dried until constant weight, and the to the root dry weight (RDW) was determined similar the SDW and also expressed in g per pot.

S-use efficiency

The determination of the S-use efficiency was calculated according to the equation below and was expressed in mg g^{-1}.

$$\text{Estimate} = (\text{Applied Level SDW} - \text{Level zero SDW})/(\text{Level Applied})$$

SPAD index, nitrogen and sulfur in shoot

The SPAD index reading was obtained for the newly expanded leaves using a Minolta Co chlorophyll meter (Minolta 1989). To obtain the mean index, 10 readings per pot were performed. Additionally, SDW from each experimental plot were ground in a Willey mill to determine N and S concentration as described by Malavolta, Vitti, and Oliveira (1997).

For the determination of N concentration, 0.1 g was weighed in the digestion tube, and 3 mL of sulfuric acid (H_2SO_4) and catalyst salts were added. The samples were put into a digester block in a ventilated flue for 4 h in order to obtain the ammonium sulfate [$(NH_4)_2SO_4$] compound. Then, the temperature was gradually increased up to 350°C. Sodium hydroxide (NaOH) (10 mol L^{-1}) was added to the samples. Then, they were transferred to a micro-distiller, where they were distilled and fixed in 10 mL of 2% boric acid (H_3BO_3) solution in the presence of green bromocresol and methyl red indicators until 40 mL of the ammonium borate solution ($NH_4H_2BO_3$) was obtained. The samples were then titrated with a 0.1 N hydrochloric acid solution (HCl) until the color changed. The N concentration was expressed in g kg^{-1} of SDW (Raij et al. 2001).

For the determination of S concentration, 0.5 g of SDW was weighed into a digestion tube and digested in nitric-perchloric acid (ClH_2NO_7) in a 4:1 ratio in a digester block in a ventilated flue. Subsequently, the S concentration was quantified by the turbidimetry of barium chloride ($BaCl_2{\cdot}H_2O$). The S concentration was expressed in g kg^{-1}. The relationship between N and S (N: S) concentration was calculated according to the concentration in the forage.

Soil chemical analysis

After the end of experiment, the potting soil was collected to determine inorganic sulfate-S ($S\text{-}SO_4^{2-}$) and pH according to the methodology described by Raij et al. (2001). The determination of $S\text{-}SO_4^{2-}$ was performed by turbidimetry, using the calcium phosphate extracting solution of $Ca(H_2PO_4)_2$ (0.01 mol L^{-1}). For this, 10 cm^3 of soil was collected and transferred to plastic flasks, and then 25 mL of the extractive solution and 0.25 g of activated charcoal were added and shaken. After stirring, the sample was filtered, and then 10 mL of the filtered solution and 1 mL of the acid solution (20 mg L^{-1} of S) were pipetted and placed in plastic flasks. Subsequently, 0.5 g of barium chloride ($BaCl_2 \cdot 2H_2O$) was added and quantified with spectrophotometer. The inorganic $S\text{-}SO_4^{2-}$ was expressed in mg kg^{-1}. For determination of soil pH, 10 cm^3 of soil was collected and transferred to plastic flasks. Then, 25 mL of calcium chloride solution ($CaCl_2$) (0.01 mol L^{-1}) was added, and it was allowed to rest for 15 min. The soil pH was measured through a potentiometer by immersing its electrodes into the suspension of sample.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the F-test at $p \leq 0.05$. When significant, the qualitative parameters were submitted to the Tukey test ($p \leq 0.05$) and the quantitative parameters were submitted to regression analysis. All data statistical analysis was performed using routines developed in the free software R (R Core Team 2018).

Results

Growth and shoot and root dry weight yield

The SDW yield of the first cut showed an isolated effect for S rates. The supply of 50 mg kg^{-1} S was sufficient to achieve a maximum yield of 26.0 g per pot, representing an increase of 9% in relation to the control (absence of S fertilizer application) (Figure 1a). In the second and third cut, the SDW indicated interactions between the S levels and sources (Figure 1b-c). The GY, GI and ESPO sources showed a similar behavior, with the 50 mg kg^{-1} level being sufficient for a maximum yield of 33.0 g per pot in the second cut. In the third cut, the SDW achieved a maximum yield of 35.0 g per pot, with the application of 50, 100 and 100 mg $kg^{-1}$to the GY, GI and ESPO sources, respectively. With the ESPA source, in the second and third cuts, the SDW was 28.6 and 28.3 g per pot and increased 30 and 58% in relation to the control, respectively.

The control had the lowest SDW yield in all cuts, especially in the third cut. In the second and third cuts, the control was lower by 52% and 131% when compared to the maximum yield of the GY, GI, and ESPO sources, while the ESPA was less by 18% and 69% in relation to the other sources, respectively. The RDW yield showed an interaction of S rates and sources (Figure 1d). The GI source achieved maximum a yield of 44.2 g per pot at the 115.0 mg kg^{-1} level, while the ESPO reached

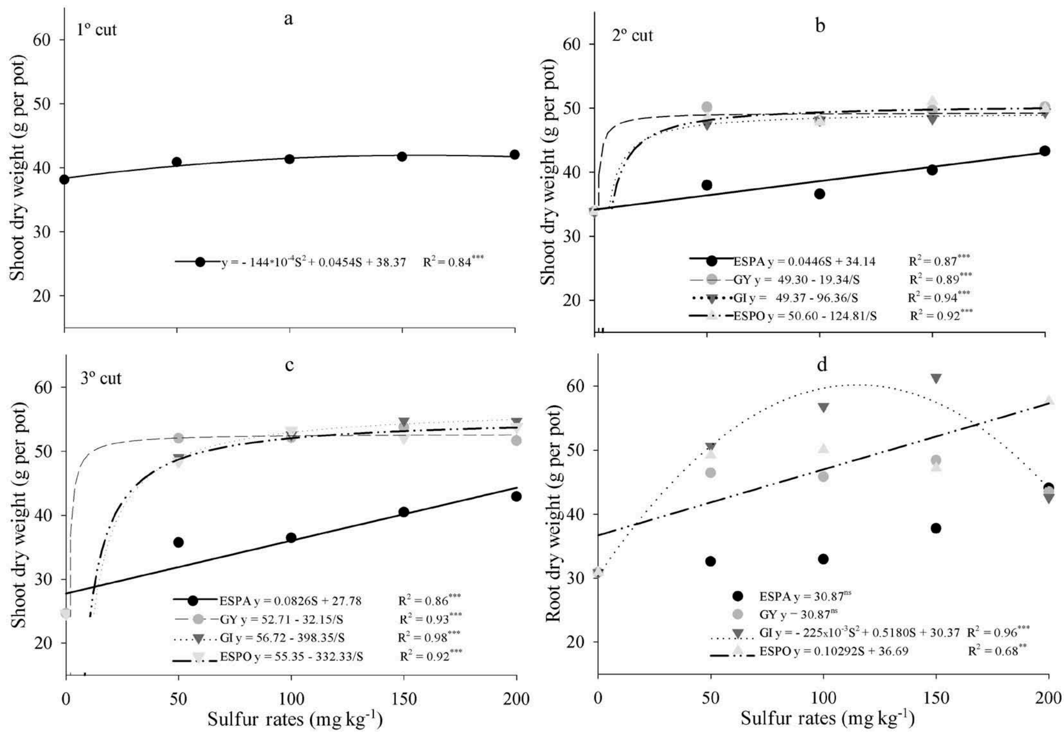

Figure 1. Isolated effects according to the levels for shoot dry weight in the first harvest (a). Interaction effect for the shoot dry weight (SDW) in the second (b) and third cuts (c). Interaction effect for the root dry weight (d) of 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; -**p* ≤ 0.05; **- *p* ≤ 0.01; *** – *p* ≤ 0.001.

41.3 g per pot at the highest level. The tiller numbers showed no significant differences in the first cut, and had a mean of 20 tillers per pot.

In the second and third cuts, an interaction effect was observed between the S rates and sources (Figure 2a-b). The GY, GI and ESPO sources had similar behaviors, reaching maximum and constant yield of tillers per pot with 50 and 100 mg kg^{-1} application, respectively, for the second and third cuts. The values obtained for each cut were on average 22 and 35 tillers per pot. The ESPA source was not significantly different in the second cut. In the third cut, values reached 28.5 tillers per pot, increasing 119% in relation to the control.

In the control, the S limitation was associated to the lower tiller numbers in the second (−66%) and third cuts (−234.8%), when compared to the maximum yield of tillers verified for the GY, GI and ESPO sources, which affected the SDW yield of the 'Mombaça' grass (Figure 1b-c).

The leaf:stem ratio presented an isolated effect for S rates and sources in the first and second cuts. The highest leaf:stem ratio was at the rate of 115 mg kg^{-1} of S for both cuts, registering increases in leaf number superior to the control of 6 and 11%, respectively (Figure 3a). As for the S source effect, in the first cut the fertilizer application with the GY e presented the highest average, and was significantly higher than the ESPA (by 5%) and not differing from the GI and SEPO (Figure 3b). In the second cut, the GY, GI, and ESPO sources did not differ and had averages significantly larger than the ESPA (by 12%).

In the third cut, the leaf:stem ratio showed interaction effects between S rates and sources (Figure 3c). The GY, GI, and ESPO presented similar behaviors and the maximum value was on average 2.1 for leaves:stem, but the rates differed among sources, and were 50 mg kg^{-1} for GY, 70 mg kg^{-1} for GI, and 100 mg kg^{-1} for ESPO, respectively, while the ESPA source had no significant effect.

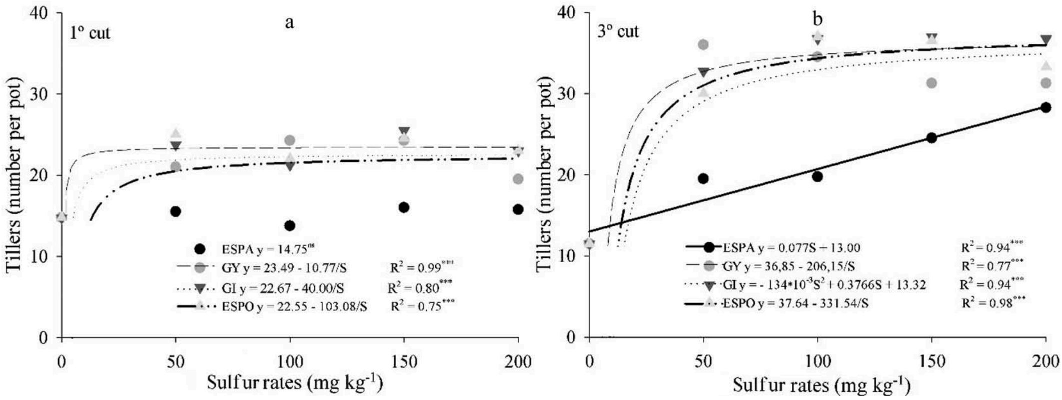

Figure 2. Interaction effect for the number tillers in the second (a) and third cuts (b) of 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; -*$p \leq 0.05$; **- $p \leq 0.01$; *** – $p \leq 0.001$.

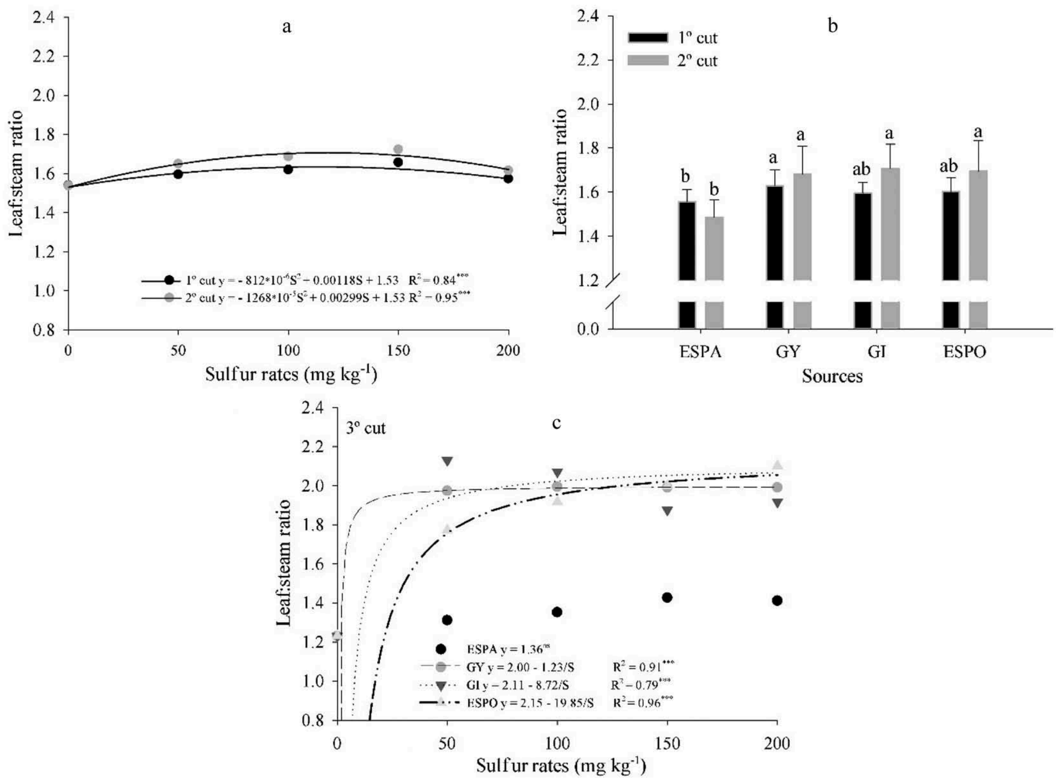

Figure 3. Isolated effects according to the rates for leaf:stem ratio in the first and second cuts (a). Interaction effects for the leaf:stem ratio in the third cut (b). Isolated effect according to the sources for leaf:stem ratio in the first and second cuts (c) of 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; –*$p \leq 0.05$; **- $p \leq 0.01$; *** – $p \leq 0.001$. Vertical bars represent the standard deviation (n = 4).

The SPAD index showed interaction effects of S rates and sources (Figure 4a–c). The SPAD index average values associated with the maximum yields of SDW were 42, 49, and 43 for the GY, GI and ESPO, respectively, and 32, 34, and 28 for the ESPA, respectively (Figure 4a-c). Visual symptoms of S deficiency were observed in the second and third cuts for the control, with values of 20 and 16 SPAD units, respectively. In the third cut, the ESPA showed visible symptoms of deficiency at all rates, with a generally yellowed appearance.

S-use efficiency

The S-use efficiency had significant effects only for rates in the first cut. The 50 mg kg^{-1} of S was significantly higher than the other rates, on average 130% (Figure 5a). In the second and third cuts, the S-use efficiency showed interaction S rates and sources. When compared to the rates effect, the 50 mg kg^{-1} of S application was significantly higher than the other rates, except for the second cut for the ESPA (Figure 5b-c). In relation to the sources effect within each rate, the GY, GI, and ESPO were significantly higher than the ESPA source, except in the third cut at the 200 mg kg^{-1} of S.

Nitrogen and sulfur in shoots dry weight

The N and S concentrations and the N:S ratio showed an interaction effect between the S rates and sources in the first and third cuts (Figure 6). In the first cut, the N concentration presented a tendency to increase (Figure 6a). On the other hand, in the third cut, the behavior was the opposite (Figure 6d).

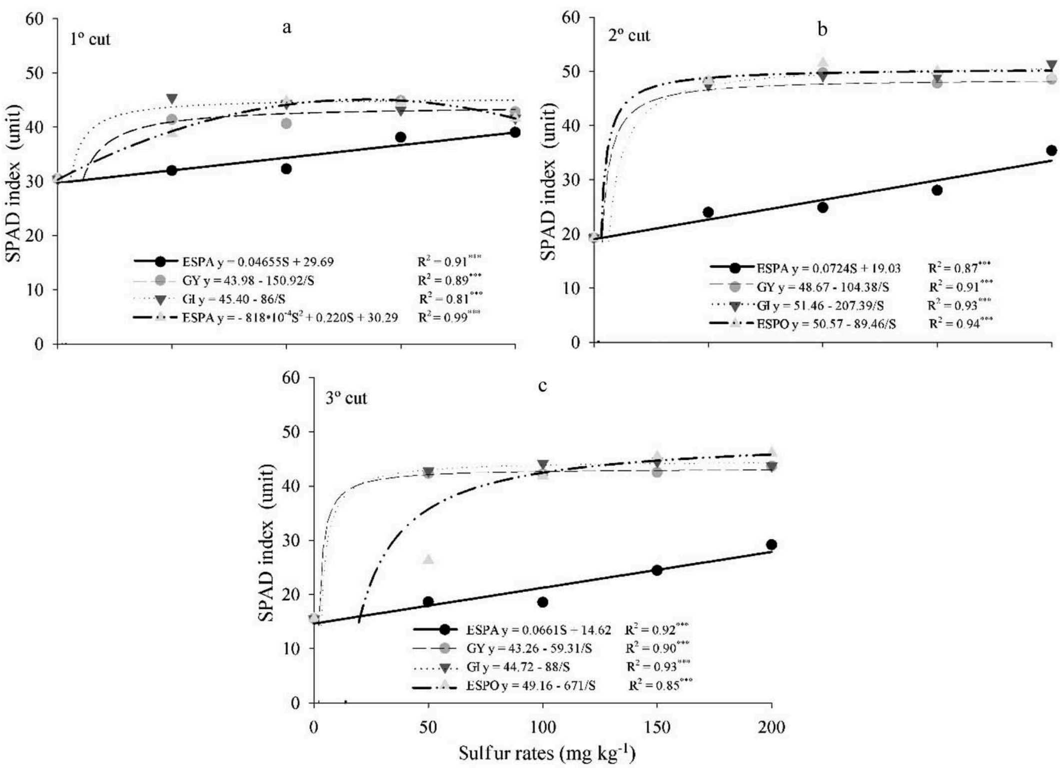

Figure 4. Interaction effect for the SPAD index in the first (a), second (b) and third cuts (c) of 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; -*$p \leq 0.05$; **- $p \leq 0.01$; *** – $p \leq 0.001$.

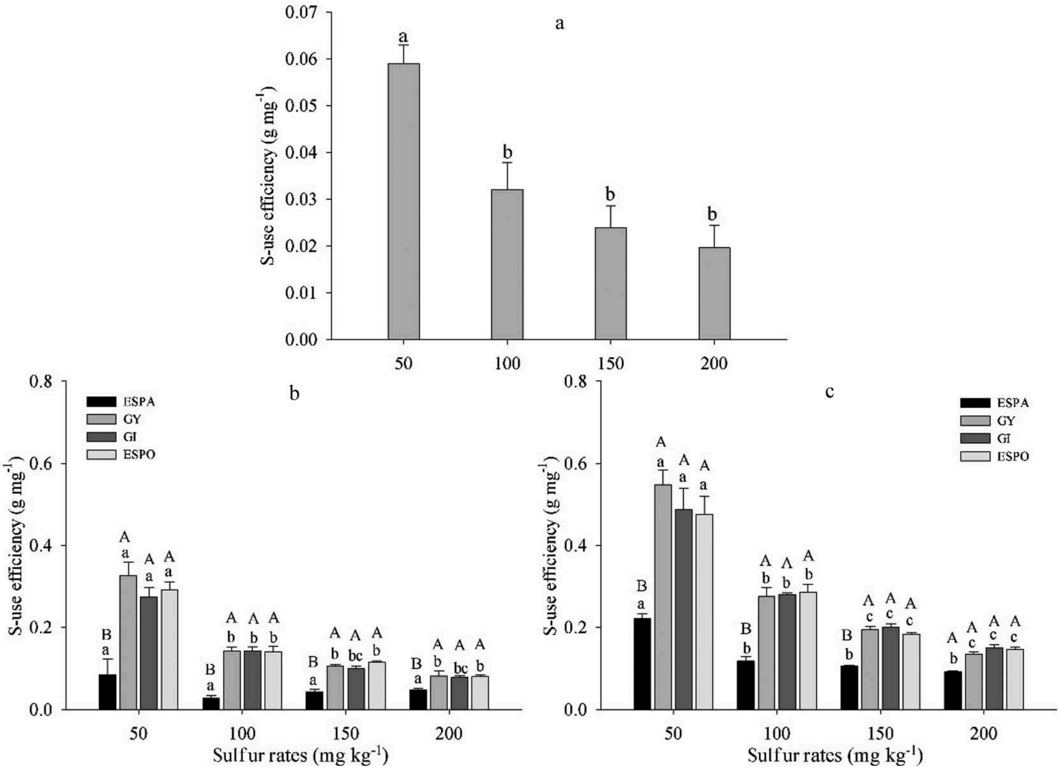

Figure 5. S-use efficiency first (a), second (b) and third cuts (c) of the 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gypsite (GI), and elemental S^0 powder (ESPO). Lowercase letters compare the effect of S rates to the same source. Upper case letters compare the effect of the sources within each rate, according to Tukey's test ($p \leq 0.05$). Vertical bars correspond to the standard deviation (n = 4).

The maximum N concentration in the first cut with the use of GY, ESPA, and ESPO sources was, on average, 24.5 g kg^{-1} at the levels of 115, 200 and 200 mg kg^{-1}, respectively. The GI source had no significant effect in the first cut. In the third cut, the N concentration associated with the maximum yield of the sources ESPA, GY, GI, and ESPO were in the following levels: 36.1, 19.9, 18.6, and 20.2 mg kg^{-1}, respectively.

The S in the first cut had similar behavior with GY and GI application, and presented a maximum concentration of 2.2 g kg^{-1} on average at the rates of 141 and 160 mg kg^{-1}, respectively. For ESPA and ESPO, the 1.9 and 2.3 g kg^{-1} of S concentrations was reached respectively, at the highest level. In the third cut, the ESPA, GY, GI, and ESPO reached concentrations of 0.9, 1.8, 1.7, and 1.8 g kg^{-1}, respectively, at the highest rate.

The results of the N:S ratio associated with the maximum SDW yield were: 16.5:1 and 38.4:1 for ESPA, 13.5:1 and 16:1 for GY, 13.5:1 and 16:1 for GI in addition to 14.5:1 and 17:1 for ESPO in the first and third cuts, respectively. The control treatment had a high N:S ratio in the third cut, with a value greater than 60:1. The SPAD index and S concentration correlated positively with SDW, while the N concentration was positively correlated with SDW in the first and negatively correlated with SDW in the third cut (Table 1). Among the variables, the one that presented the highest correlation coefficient with SDW was the SPAD index, with 93% values in the second and 91% in the third cut.

Inorganic S-sulfate and soil pH

Inorganic S-SO_4^{2-} and pH had interactions S levels and sources (Figure 7a). S-SO_4^{2-} was directly proportional to the increase in the S levels. The ESPA, GY, GI and ESPO reached the concentrations

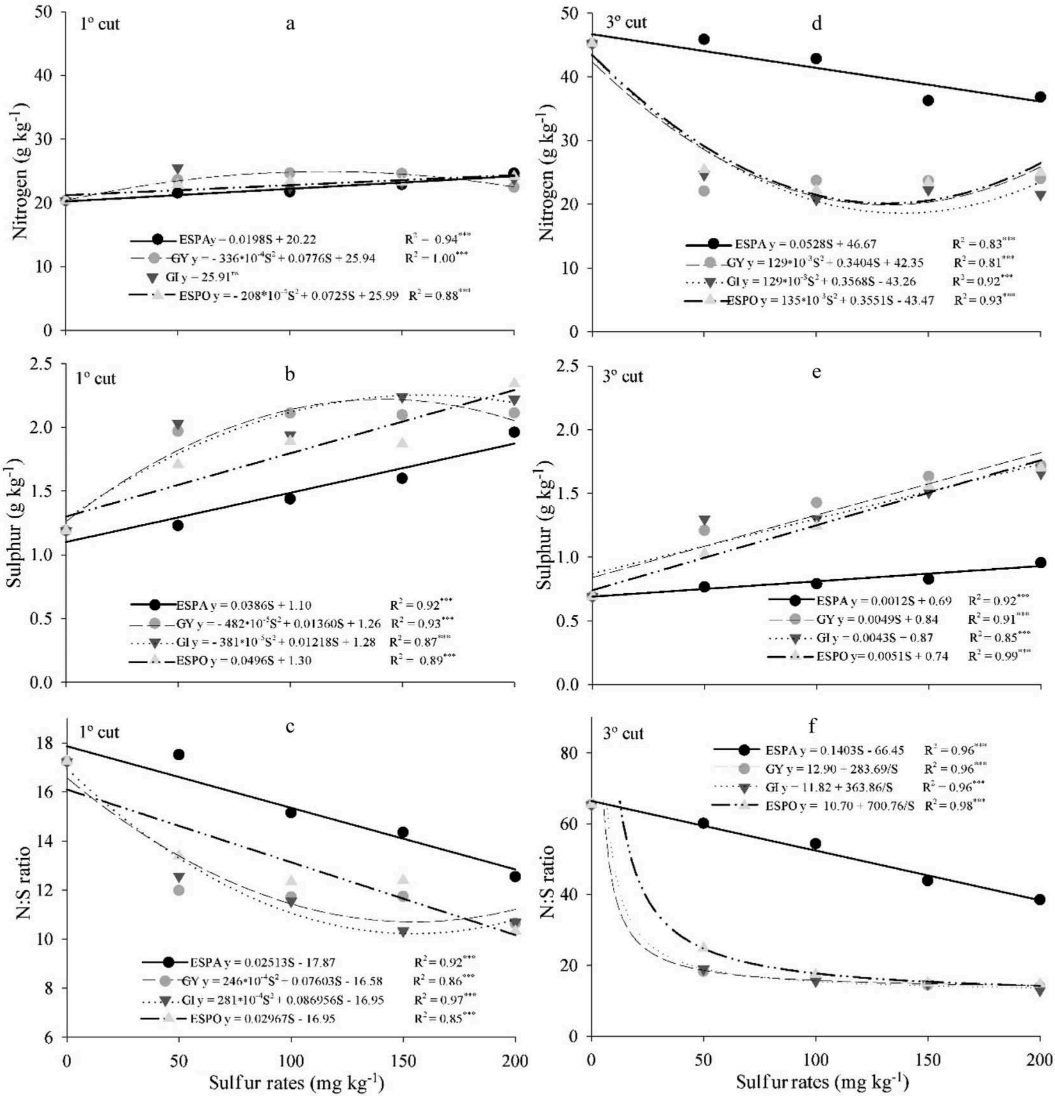

Figure 6. Interaction effect for N (a and d) and S concentration (b and e); and N:S ratio (c and f) respectively in the second and third cuts of 'Mombaça' grass in response to S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; -*$p \le 0.05$; **- $p \le 0.01$; *** – $p \le 0.001$.

of 13.7, 25.9, 27.8, and 23.8 mg kg^{-1} at the highest level, respectively. The pH had similar behavior with the GY and ESPO application, which had reductions of 6.5% and 19.0% in the highest rate, respectively (Figure 7b). The ESPA had a maximum reduction of 7.0% at the 128 mg kg^{-1} S. The GI source had no significant effect for this parameter.

Discussion

Shoot and root dry weight yield

Many areas under pasture cultivation in tropical conditions are subject to S deficiency. The proper application of this nutrient to the soil can remedy nutrient deficiencies and, consequently, result in

Table 1. Correlation coefficients between shoot dry weight (SDW), SPAD index, N and S concentration in 'Mombaça' grass in the first and third cut.

Variables	SPAD	N	S
		1st cut	
SDW	0.60***	0.42**	0.65***
		2nd cut	
SDW	0.93***	..	..
		3th cut	
SDW	0.91***	−0.93***	0.87***

** – $p \le 0.01$; ***.

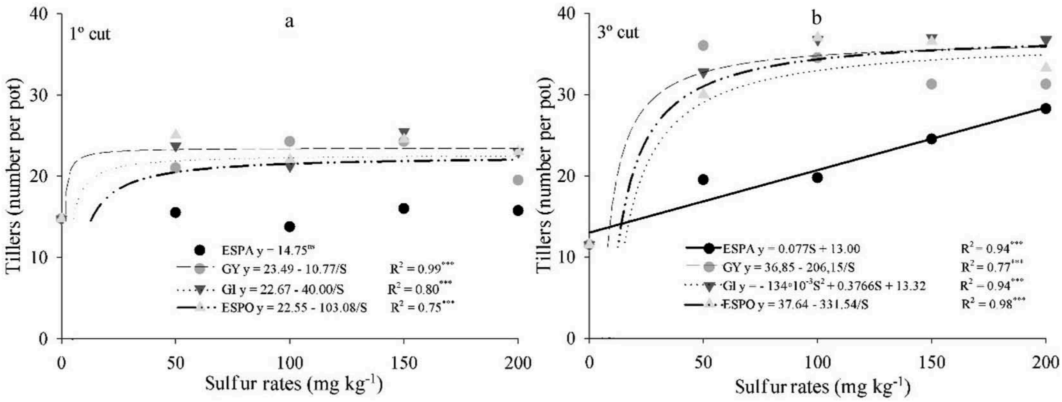

Figure 7. Interaction effect for inorganic sulfate-S, S-SO_4^{2-} (a) and pH of soil (b) after the cultivation of 'Mombaça' grass fertilized with different S rates and sources. Elemental S^0 pastilles (ESPA), phosphogypsum (GY), gibbsite (GI), and elemental S^0 powder (ESPO). ^{ns} – not significant; *$p \le 0.05$; **- $p \le 0.01$; *** – $p \le 0.001$.

increased forage yield. Increases in SDW yield (Figure 1a–c) and RDM (Figure 1d) are linked to the fact that S plays catalytic, regulatory and structural functions in plants (Capaldi et al. 2015; Ding et al. 2016), in addition to providing higher nutritional quality conferred by the adequate S concentration to the plant (Gilabel et al. 2014).

The favorable actions of S application on the three primary vegetative parameters evaluated (shoot, root, and tiller numbers) (Figures 1,2) are fundamental for optimizing the 'Mombaça' grass yield (Heinrichs et al. 2013; Lavres Júnior, Monteiro, and Schiavuzzo 2008) and assuring fast growth of the plants after cut or grazing (Schmidt and Monteiro 2015). The yield results corroborate previous studies in a greenhouse with forage, which also verified increases in the shoot, root (Custódio et al. 2005; Heinrichs et al. 2013; Lavres Júnior, Monteiro, and Schiavuzzo 2008; Schmidt and Monteiro 2015), and tiller numbers (De Bona and Monteiro 2010a; Schmidt and Monteiro 2015).

Therefore, based on the present data, the S application stimulated the increase of forage yield, as much by plant growth as by the greater tiller numbers in an area. The increase observed in the leaf: stem ratio due to S application (Figure 3a–c) provided higher forage quality. This was because the leaves had a higher nutrient concentration, which increased the forage supply and the voluntary consumption through grazing by animals (De Bona and Monteiro 2010A; Schmidt and Monteiro 2015).

The SPAD index was an adequate indicator to evaluate the nutritional status of 'Mombaça' grass, and showed a high correlation coefficient with the SDW (Table 1), making it possible to detect changes in the readings caused by the S supply. S deficient symptoms were verified with values lower than 25 SPAD index units in the second and third cuts (Figure 4b–c). The values above 40 SPAD index units were associated with maximum 'Mombaça' grass yield. Similar results were obtained by Schmidt and Monteiro (2015) with the same forage.

These results could be related to the reduction of chlorophyll content due to S deficiency, characterized by the yellowing of the leaves that is initiated by the younger leaves (Imsande 1998; Lunde et al. 2008). An increase in the SPAD index reading could be related to the importance of S in increasing the photosynthetic capacity of plants (Pandey, Naaz, and Ansari 2009). This would improve the efficiency of the ferredoxin-thioredoxin system, which regulates the functioning of enzymes in C fixation in addition to several other processes in the chloroplast (Crusciol et al. 2013; Marschner 2012; Taiz et al. 2017).

The results also showed that the best use efficiency was obtained with the 50 mg kg^{-1} of S, and decreased with increasing S levels (Figure 5). In the second and third cuts, the GI, GY, and ESPO did not differ among themselves and were higher than ESPA (Figure 5b–c). Thus, the processes in the soil associated with S availability had great influence on the granulometry of the ESPA fertilizer.

Shoot dry weight yield in function of the sulfur sources and cut

The SDW yield, tiller number, leaf:stem ratio, SPAD index, and S use efficiency showed differences between the cuts. The second and third cuts, the 'Mombaça' grass showed a higher response to S, whereas the control had the opposite behavior (Figure 1a–c). This can be explained by the average S concentration in the initial soil analysis and the low SOM content. It is possible that it occurred due to the deposition and some mineralization in the initial period of the experiment, which may have provided sufficient S to explain the differing performance of the first cut. However, for the successive cuts, the S supply was insufficient to meet the crop demand, resulting in the deficiency of this crop nutrient at the second and third cuts (Figure 6b–e).

The S supplied in the form of S^0 or SO_4^{2-}, in addition to the granulometry of fertilizer (ESPA and ESPO) affected the increase of SDW, tiller number, SPAD, and S use efficiency (Figures 1,2,4 and 5), especially for ESPA, which presented less response in relation to the other sources. Possibly, this differentiated response is associated with the granulometry of source, since the ESPA fertilizer is in a granular form, while the other sources are in a powder form. This hypothesis is supported by the fact that the ESPA and ESPO are composed by the same form of elemental S (S^0) and differed statistically from each other. Other researches also report the effect of physical and chemical properties on S availability (Sun et al. 2017). Wen et al. (2001) observed that the oxidation of S varies as a function of temperature and granulometry, presenting oxidation rates after ten weeks of application of 11, 26, and 56% for S^0 powder and 1, 4, and 8% levels for granulated S^0 at 5, 10, and 20°C, respectively.

In view of that information, it was verified that the availability of S^0 could be increased by formulations designed to reduce the particle size and increase the surface area exposed to microbial activity (Degryse et al. 2018; Grant, Mahli, and Karamanos 2012). In addition, the sources that are composed of S^0 are subordinated to the abiotic and biotic factors that affect microbial activity that will influence its supply to the plant during the crop cycle (Brahim et al. 2017; Degryse et al. 2018).

Nitrogen and sulfur in shoots

A synergistic effect was observed between N concentrations and increasing S rates in the first cut (Figure 6a). This effect may be linked to higher synthesis of cysteine and methionine amino acids, which are important for tertiary structure determination and protein synthesis, generating a higher N requirement for protein formation. Furthermore, S favors the activity of nitrate reductase (N-NO_3) and glutamine synthetase enzymes, which are part of N reduction and assimilation in amino acids (De Bona and Monteiro 2010b; Grant, Mahli, and Karamanos 2012; Heinrichs et al. 2013; Salvagiotti and Miralles 2008). Regarding the third cut, N concentration reduced with S fertilizer application (Figure 6d). This is probably because the higher 'Mombaça' vegetative grass growth caused a dilution effect as verified by Heinrichs et al. (2016).

The S concentrations associated with the maximum SDW yield in the first and third cuts were in the range of 1.5–2.2 g kg^{-1}, for the ESPO, GI, and GY, which was considered adequate for 'Mombaça' grass nutrition (Malavolta, Vitti, and Oliveira 1997). The control and ESPA in the third cut had concentrations below the critical limit of culture deficiency, which was reflected in a lower yield and nutritional imbalance (Figures 1c, 6d–f). It is important to emphasize that successive cultivation without the replacement of this nutrient can deplete the natural organic S, aggravating the deficiency of nutrient in the soil (Degryse et al. 2018; Schmidt and Monteiro 2015).

The maximum SDW yield associated with the N:S ratio revealed the best relation of these nutrients for the 'Mombaça' grass yield, with variations between 13.5:1 and 17:1 for GY, GI and ESPO in both cuts analyzed, which corresponded to the 14:1 ratio reported for plants of Poaceae family (Gramineae) (De Bona and Monteiro 2010b). The control and ESPA showed a high N:S ratio in the third cut, approximately 60:1 and 38:1, respectively, which characterizes a metabolic imbalance of the plant (Figure 6c–f). This imbalance may also be associated with the lower development and growth of forage.

Crusciol et al. (2013) reported that the insufficient S supply results in imbalance of the N:S ratio in plants, consequently, N accumulation in the non-protein form can occur, especially the forms of NO_3^- and N-organic soluble (Haq and Carlson 1993), which results in lower nutritional quality of grass and reduction of plant growth.

Inorganic S-sulfate and influence on soil pH

The S-SO_4^{2-} content in the soil at the beginning of the research was 7.0 mg kg^{-1}. After the three harvests in the control, the values decreased dramatically, ranging from 1.1 to 4.3 mg kg^{-1}, representing decreases ranging from 42- to 81% of the soil original availability (Figure 7a), which resulted in nutrient deficiency in the crop. On the other hand, with the S application, the S-SO_4^{2-} was above the level considered sufficient for demanding crops (10 mg kg^{-1}) for all sources studied (Figure 6a). Kopittke, Dalal, and Menzies (2016) reported that the continuous organic S mineralization is insufficient to meet the needs of crops, especially in soils with low SOM content. The results of this study show that nutrient application is fundamental to maintain soil availability, even in conditions of adequate initial availability.

A soil pH reduction was observed after three successive cuts of 'Mombaça' grass, which can be attributed to the higher base extraction, especially with increased yield, as verified by Ahmed et al. (2017). In particular, for the ESPO, was related to a marked reduction of pH due to its physical and granulometric properties, which were related to S^0 oxidation that released SO_4^{2-} and H^+ in the soil (Boaro et al. 2014).

Conclusion

In general, the results showed that the S-use efficiency with the GI, GY, and ESPO sources was obtained with the supply of 50 mg kg^{-1} of S, and this value was enough to optimize the yield of 'Mombaça' grass and promote a better quality for the nutritional status of the forage. The ESPA source had an S leaf concentration that was below the adequate range and, consequently, resulted in lower yield values in relation to the other S sources evaluated. The SPAD index in the newly expanded leaves was shown to be an indicator of SDW yield and could be used as a pre-diagnosis of S level in 'Mombaça' grass. The soil processes associated to the S availability had a great influence on the granulometry, which drove the yield SDW and S use efficiency with the ESPO in relation to the ESPA. The S availability in the soil was reduced substantially in the control, which caused S deficiency symptoms in the leaves of 'Mombaça' grass, nutritional imbalance of N:S ratio and lower yield of SDW in the second and third cuts, evidencing the need to S supply in consecutive crops.

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