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Absence of Sulfur Fertilization at Establishment in *Urochloa brizantha* Cultivars

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Abstract

Sulfur-containing fertilizers increase production costs, which leads to low utilization of this nutrient. Thus, evaluating how the absence of sulfur influences the early development of *Urochloa brizantha* is essential. Study was conducted in a greenhouse at the Federal University of Rondonópolis in a completely randomized design, with six treatments in a 3 × 2 factorial scheme, and eight replications. Three cultivars of *U. brizantha* (Marandu, Xaraés and Piatã) were evaluated under two fertilization strategies: with or without sulfur fertilization. Sufur presence increased the number of leaves and forage mass, in which cultivar Xaraés presented the greatest means. Piatã was the cultivar most sensitive to sulfur deficiency at establishment, which reduced forage mass, number of leaves and number of tillers by 42%, 32%, and 45%, respectively. Despite these differences between cultivars, sulfur efficiently increased the forage yield. Sulfur fertilization increased the concentrations of nutrients in the plants without significantly affecting the uptake of nitrogen, phosphorus, potassium, calcium and magnesium. Sulfur omission resulted in increased phosphorus uptake in all grass. In contrast, Marandu grass exhibited the greatest reduction in sulfur uptake. Therefore, the use of sulfur in the fertilization of grasses is recommended, it is important to evaluate the responses of each cultivar to better adjust the fertilization management.

Keywords: *Brachiaria brizantha*; sulphur requirement; pasture implantation; simple superphosphate

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1. Introduction

Fertilization in soils with low natural fertility, such as Latosols, is an indispensable practice to prevent pasture degradation and ensure the proper development of forages. Among the essential nutrients for forage development, sulfur plays an essential role in plant physiology. Deficiency in this nutrient can reduce nitrogen assimilation [1], which restricts plant development [2]. Sulfur deficiency can impair nitrogen assimilation by limiting the synthesis of sulfur-containing amino acids, though the extent of this effect depends on species, growth stage, and nutrient availability.

Due to sulfur participate in different physiological process in the plants, studies have been conducted to better understand the effects of this nutrient in plants metabolism [3]. With that, an inadequate sulfur supply may restrict the establishment of grasses. In tropical grazing systems, the management of fertilization is primarily supplying limestone, and macronutrients, such as nitrogen, phosphorus and potassium. This imbalance in nutritional management can compromise the productive potential of grasses [4]. Thus, study the effects of sulfur fertilization on forage growth and morphological characteristics in tropical grasses is important.

The omission of sulfur in fertilization can reduce the forage mass of *Urochloa brizantha* cv. Marandu by 66% [5]. However, similar results with such restrictions are not observed because the mineralization of organic matter is an important source of sulfur. For this reason, other studies indicate that there is no yield restriction on grasses due to the omission of sulfur [6,7]. Other studies have shown positive interactions between nitrogen and sulfur in maximizing the production of shoot biomass and in the nutritional recovery of grasses [8,9].

The inclusion of sulfur in the nutritional management of pastures, implies an increase in production costs, and requires evaluation from a cost-benefit perspective. The application of phosphorus, in the form of single superphosphate, or nitrogen, in the form of ammonium sulfate, usually involves the need for sulfur in the forage; however, these fertilizers are more expensive than monoammonium phosphate and urea, respectively.

Therefore, studies are needed to measure the impact of sulfur absence can restrict the development of grasses to obtain a parameter that is comparable to the increase in cost. Therefore, the objective of this research was to verify whether the absence of sulfur limits establishment of cultivars of *Urochloa brizantha* (syn. *Brachiaria brizantha*) and whether there is a difference in nutritional requirements between the cultivars.

2. Materials and Methods

The study was carried out in a greenhouse at the Federal University of Rondonópolis in a completely randomized experimental design in a factorial 3 × 2 scheme, with six treatments and eight replications. The treatments consisted of three cultivars of *U. brizantha* (Marandu, Xaraés and Piatã) and two establishment fertilization strategies: with or without sulfur. Each experimental unit consisted of a 3.0 dm^3 pot containing three plants. The soil used was a clayey Oxisol (Table 1) collected from the 0 to 0.20 m layer, which was sieved and transferred to the pots. The initial soil sulfur content was very low (Table 1), well below the recommended level of 13.1 mg dm^{-3} [10], which justifies the need to investigate the effects of sulfur deficiency in this study.

Table 1. Particle size and chemical characterization of an Oxisol from the Cerrado.

pH	P	K	S	Ca + Mg	Al + H	CEC	V	M	O.M	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}			cmolc dm^{-3}			%			g kg^{-1}		
6.0	3.4	119.0	1.0	4.3	1.7	6.3	73	0.0	23.0	575	50	375

CEC: cation exchange capacity; O.M.: organic matter; V: base saturation; M: aluminum saturation.

At sowing, the plants were fertilized with 300 mg dm^{-3} phosphorus. The treatments with and without sulfur were fertilized with single superphosphate (18% P_2O_5, 8% S), resulting in a dose of 24 mg dm^{-3} of sulfur, and triple superphosphate (41% P_2O_5), respectively (Figure 1). Twenty seeds were sown per pot, and after emergence, thinning was carried out leaving three plants per pot. After thinning, nitrogen fertilization was applied at a dose of 100 mg dm^{-3}. The pots were irrigated daily, increasing the humidity to the maximum water retention capacity in the soil, which was determined according to [11]. No

pest or disease issues were observed during the experiment, and thus no control measures were necessary, ensuring that the results were not influenced by these biotic factors.

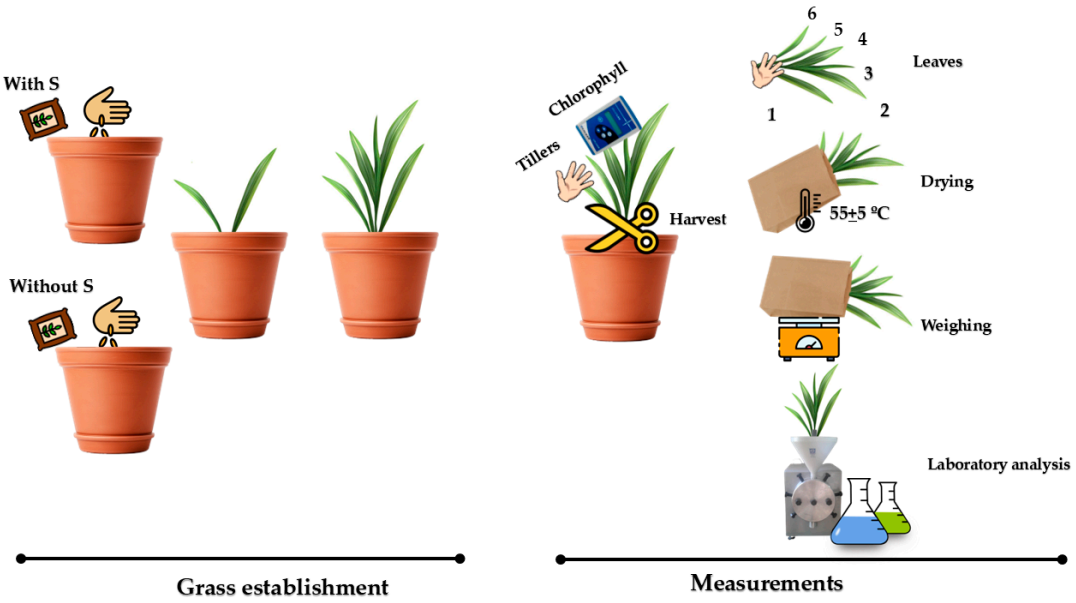


Figure 1. Schematic representation of all experimental phases in the study on the absence of sulfur fertilization during the establishment of *Urochloa brizantha* cultivars.

Before plants were harvested, their height was measured using a measuring tape scaled in centimeters, and the number of tillers was counted. The chlorophyll index was measured in half-thirds of three newly expanded leaves using a chlorophyllometer (model ClorofiLOG/CFL 1030) (Falker, Porto Alegre, Brazil). The cutoff for the evaluation of the experimental variables occurred when the plants reached 30 cm in height, which occurred thirty-five days after sowing. In harvest, plants were cut with a residue height of 15 cm above the ground.

After being cut, the number of leaves present in the forage harvested was counted. It was not necessary to perform morphological separation, as there were only leaf blades presented in the material harvested. Samples from each pot were separated and placed in an oven with forced air circulation set to a constant temperature of 55 ± 5 °C until the dry weight was constant. The leaves were ground in a knife-type mill, and the total concentrations of nitrogen, phosphorus, potassium, calcium, magnesium and sulfur were determined as described by [12]. Nutrient concentrations were expressed in g kg^{-1}, and through the product with the forage mass, nutrient uptake (g pot^{-1}) was obtained:

$$\text{Nutrient uptake} = \text{nutrient concentrations} \times \text{forage mass}$$

The dry mass of each tiller was estimated by the ratio of the forage mass to the number of tillers. The dry mass of each leaf blade was obtained by dividing the dry mass of the leaf blades by the number of leaves. By dividing the number of leaves per tiller by the interval, in days, between seedling emergence and plant cutting, the leaf appearance rate was estimated.

The statistical model used was as follows:

$$Y_{ijk} = \mu + C_i + A_j + (C \times A)_{ij} + e_{ijk}$$

where Y_{ijk} represents the value observed for each experimental unit; μ represents the general average; C_i represents the effect of *Urochloa brizantha* cultivar; A_j represents the

fertilization effect; $(C \times A)_{ij}$ represents the interaction between factors; and e_{ijk} represents the experimental error associated with the sampling unit.

Variables in which the isolated effect of fertilization or the interaction between cultivar and fertilization was identified, the degree to which the absence of fertilization changed these variables was estimated, with grass fertilized with sulfur as a reference. The following equation was used:

$$\text{Reduction or increase} = [(\overline{x}\,\text{NF} - \overline{x}\,\text{F})/\overline{x}\,\text{F}] \times 100$$

where $\overline{x}$ NF = average not fertilized; $\overline{x}$ F = average fertilized.

For statistical analysis, analysis of variance was performed to identify isolated effects or interactions between factors, and then the Tukey test was applied. Both tests were performed at a 5% probability of error.

3. Results

Sulfur fertilization influenced the number of tillers, number of leaves, forage mass, concentration of macronutrients, and uptake of P, Ca, Mg, and S (Table 2). There was an interaction effect between grasses and sulfur fertilization only for the number of tillers and nitrogen, potassium, magnesium and sulfur concentration (Table 2). The mass of each leaf, mass of each tiller, the chlorophyll index and N uptake were not affected by any of the sources of variation (Table 2).

Table 2. Synthesis of the analysis of variance for the effects of grasses, sulfur fertilization and the interaction between grasses and fertilization.

Variable	Grass (G)	Fertilization (F)	G × F	SEM
Productive, structural and morphogenic variables				
Forage mass	0.0265	0.0014	0.3994	0.277
Number of tillers	0.1877	0.0001	0.0185	1.708
Number of leaves	0.0340	0.0031	0.6207	3.250
Mass of each leaf	0.2118	0.7533	0.5946	0.005
Mass of each tiller	0.6427	0.9249	0.1803	0.011
Leaves per tiller	0.5066	0.7836	0.1301	0.147
Leaf Appearance Rate	0.5067	0.7837	0.1302	0.005
Nutritional variables				
N concentration	0.0002	0.0001	0.0075	0.634
P concentration	0.0013	0.0001	0.0807	0.154
K concentration	<0.0001	<0.0001	<0.0001	0.168
Ca concentration	0.0009	0.0149	0.5316	0.235
Mg concentration	<0.0001	0.0248	0.0001	0.023
S concentration	0.0002	<0.0001	0.0026	0.116
N uptake	0.6418	0.0797	0.3695	5.540
P uptake	0.0162	0.0245	0.0956	0.409
K uptake	0.1935	0.7902	0.4123	2.920
Ca uptake	0.0738	0.0298	0.3970	2.008
Mg uptake	0.0356	0.0014	0.2086	0.627
S uptake	0.2230	0.0000	0.6891	0.547
Chlorophyll Index	0.0116	0.5547	0.1698	1.237

SEM: standard error of the mean.

Regardless of cultivar, sulfur fertilization resulted in a greater number of leaves, forage mass and uptake of secondary macronutrients (Ca, Mg and S) (Table 3). On average, 77% of the leaves emitted by the sulfur grasses were observed in the sulfur-free grasses, whereas 74% of the forage mass was produced.

Table 3. Means of the morphological, productive and nutritional variables of *Urochloa brizantha* as a function of grasses and sulfur fertilization at establishment.

Variable	Grasses			Fertilization	
	Marandu	Xaraés	Piatã	Without S	With S
Productive, structural and morphogenic variables					
Number of leaves (leaves pot^{-1})	50	51 a	39 b	41 B	53 A
Forage mass (g pot^{-1})	3.4 b	4.3 a	3.3 b	3.1 B	4.2 A
Mass of each sheet (g)	0.07 a	0.08 a	0.08 a	0.07 A	0.08 A
Mass of each tiller (g)	0.12 a	0.14 a	0.12 a	0.13 A	0.13 A
Leaves per tiller	2 a	2 a	2 a	2 A	2 A
LAR [1] (leaf day^{-1})	0.07 a	0.06 a	0.06 a	0.6 A	0.6 A
Nutritional variables					
Teor P (g kg^{-1})	1.4 b	1.4 b	2.2 a	2.1 A	1.2 B
Teor Ca (g kg^{-1})	8.1 a	6.7 b	6.9 b	7.6 A	6.8 B
N uptake (g pot^{-1})	73 a	79 a	74 a	69 A	81 A
P uptake (g pot^{-1})	5 a	6 a	7 a	6 A	5 B
K uptake (g pot^{-1})	36 a	43 a	42 a	41 A	40 A
Ca uptake (g pot^{-1})	28 a	28 a	22 a	24 B	29 A
Mg uptake (g pot$^{-1)}$	8 a	8 a	6 a	6 B	9 A
S uptake (g pot^{-1})	5 a	5 a	6 a	1 B	9 A
Chlorophyll Index	42 b	43 b	48 a	44 A	44 A

[1] LAR: leaf appearance rate. Means followed by the same uppercase letter, for fertilization, and by the same lowercase letter, for grasses, did not differ significantly from each other, according to Tukey's test ($p > 0.05$).

Greater P uptake was identified for grasses established without sulfur (Table 3). Sulfur fertilization did not affect the mass of each leaf, mass of each tiller, number of leaves per tiller, or leaf appearance rate (Table 3). All macronutrient uptakes were altered by sulfur fertilization, with exception of nitrogen and potassium (Table 3).

All grasses, regardless of fertilization, had the same macronutrient uptake, mass of each leaf, mass of each tiller, number of leaves per tiller and leaf appearance rate (Table 3). Xaraés and Piatã grasses differed in terms of the number of leaves and forage mass (Table 3). The highest chlorophyll index was observed in Piatã grass (Table 3).

There was no difference in number of tillers on Marandu grass fertilized with or without sulfur (Table 4). The Xaraés and Piatã grasses presented the greatest number of tillers when fertilized with sulfur (Table 4). When fertilized with sulfur, all grasses had the same number of tillers, while in the absence of sulfur, Piatã grass had the lowest number of tillers (Table 4).

The highest levels of calcium, phosphorus (Table 2) and potassium (Table 3) occurred in cultivars cultivated without sulfur. Marandu was the only grass in which the grasses fertilized with and without sulfur had the same nitrogen content because, for Xaraés and Piatã, the highest nitrogen content was observed in grasses without sulfur fertilization (Table 3). For the three cultivars, there was a lower sulfur content due to the absence of sulfur (Table 3).

All grasses presented the same reduction in the number of leaves (23%) when established without sulfur. However, the smallest reduction in the number of tillers, due to the omission of sulfur, occurred in Marandu grass (Figure 2). The greatest reduction in

forage mass occurred on Piatã grass (Figure 2). Owing to the omission of sulfur, all grasses presented an increase in phosphorus uptake, and there was a increase in the uptake of this nutrient for Marandu and Xaraés grasses (Figure 3). The greatest reduction in S uptake occurred in Marandu grass (Figure 3).

Table 4. Number of tillers and nutrient contents in cultivars of *Urochloa brizantha* subjected to different sulfur fertilization strategies at establishment.

Fertilization	Marandu	Xaraés	Piatã
		Tillers (number pot^{-1})	
With S	29 aA	36 aA	34 aA
Without S	27 aA	26 aB	19 bB
		N concentration (g kg^{-1})	
With S	20.8 aA	16.6 bB	20.1 aB
Without S	21.0 bA	20.5 bA	26.8 aA
		K concentration (g kg^{-1})	
With S	9.45 bB	8.45 cB	10.9 aB
Without S	12.0 bA	12.0 bA	17.0 aA
		Mg concentration (g kg^{-1})	
With S	2.6 aA	1.9 cB	1.9 cB
Without S	2.4 aB	2.0 bA	2.0 bA
		S concentration (g kg^{-1})	
With S	2.6 aA	1.8 bA	2.5 aA
Without S	0.4 bB	0.5 bB	1.1 aB

Means followed by the same uppercase letter within a column and the same lowercase letter within a row do not differ significantly from each other, according to Tukey's test at the 5% probability level.

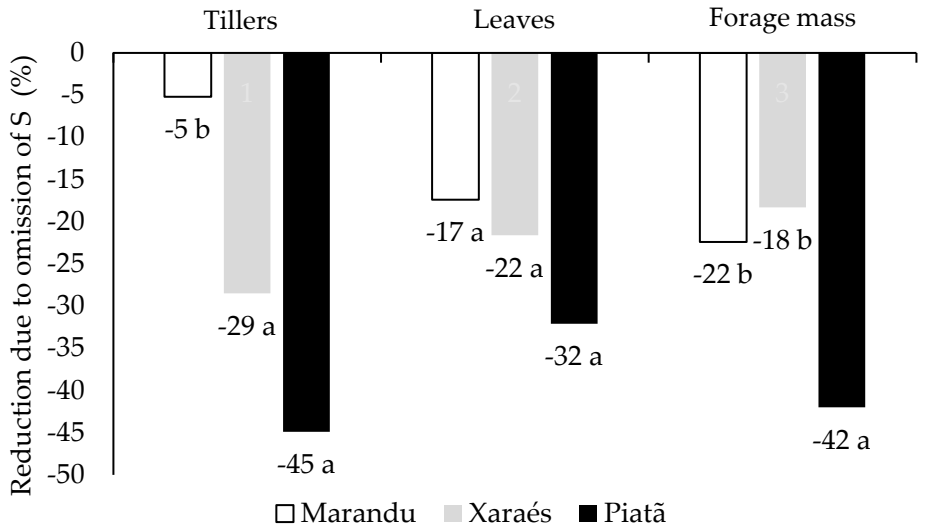


Figure 2. Reduction in the number of tillers, leaves and forage mass of *Urochloa brizantha* cultivars due to the omission of sulfur at establishment. Means followed by the same letter did not differ according to the Tukey's test ($p > 0.05$).

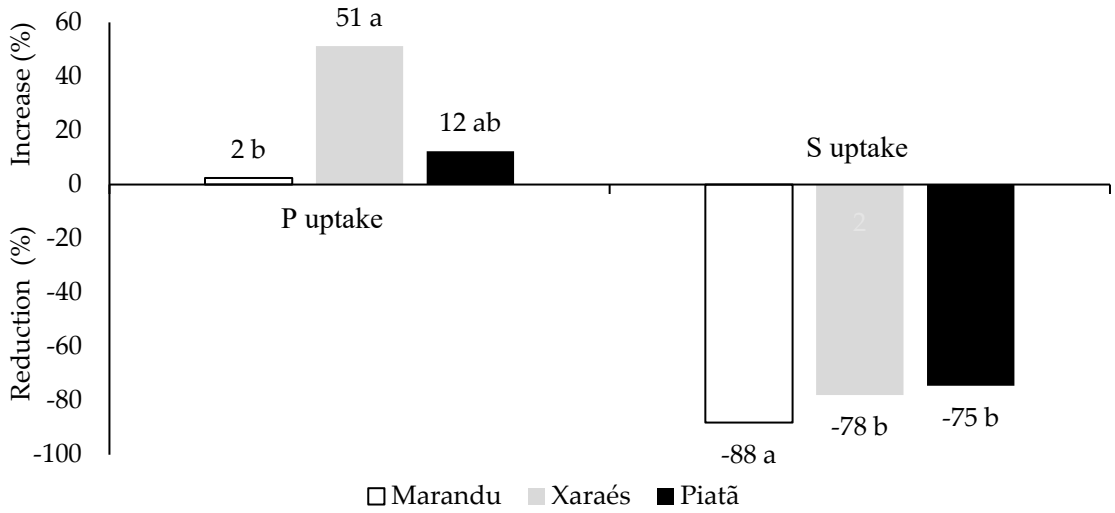


Figure 3. Percentage variation in P and S uptake by *Urochloa brizantha* cultivars under sulfur omission. Negative values indicate reductions relative to the control (with sulfur). Means followed by the same letter do not differ by Tukey's test ($p > 0.05$).

4. Discussion

Sulfur absence impaired the development of the cultivars of *Urochloa brizantha*, as the forage mass and number of leaves decreased by 26% and 23%, respectively, regardless of the cultivar. Similar results were reported by [13,14], who reported 30% and 35% reductions in the establishment of *U. brizantha* cv. Marandu and *U. brizantha* cv. Piatã, respectively. This reduction in forage mass due to the omission of sulfur resulted in a higher concentration of nutrients in the leaf tissues, mainly phosphorus, calcium and potassium.

This occurred because sulfur is essential for the synthesis of amino acids and proteins, which are fundamental processes for plant growth [15]. In addition, this delay in the development of grasses not fertilized with sulfur can be attributed to the insufficient amount of sulfur in the soil, since the sulfur content in the soil for the establishment of *Urochloa brizantha* must be greater than 13.1 mg dm^{-3} [10].

This sulfur deficiency is closely related to the characteristics of the soil used in the study. The very low sulfur content observed here is typical of Oxisols, which are highly weathered soils commonly found in tropical regions. Due to their advanced stage of pedogenesis, these soils exhibit low natural fertility and low levels of all macronutrients, including sulfur. In such soils, sulfur is primarily supplied through the mineralization of organic matter, which itself contains low amounts of this nutrient.

Although all cultivars presented a reduction in forage mass, Piatã was more affected by the greater reduction in forage mass and number of tillers. This result demonstrates greater sulfur requirements, and in the absence of sulfur fertilization at establishment, there is a greater predisposition to early degradation. As this grass has greater restrictions on forage mass, a sulfur concentration effect was identified since the content of this nutrient was greater than that in Marandu and Xaraés. This variability in the response of different cultivars to sulfur availability reinforces the observations of [10], who demonstrated the need for specific management strategies for each cultivar.

Marandu and Xaraés grasses had the lowest reduction in forage mass, which demonstrates lower nutritional requirements than Piatã grass. All these grasses had similar restrictions on leaf emission; however, the absence of sulfur did not impair the tillering of Marandu grass, which demonstrates that this cultivar was the least affected by the omission of sulfur.

Maintaining the tillering of Marandu grass in the absence of sulfur is important, as it increases soil cover and reduces the predisposition to erosion [16] and weed emergence [17]. In addition, tillering is necessary for pasture continuity [1], which points to Marandu grass as the *Urochloa brizantha* cultivar with the lowest sulfur requirement. The lower sulfur requirement of Marandu can also be observed through the nitrogen content of the leaf tissues, which was not affected by sulfur. There is a relationship between nitrogen and sulfur, so in some grasses, sulfur fertilization increases uptake [18].

The absence of sulfur at establishment resulted in lower uptake and concentration of this nutrient in all the cultivars studied, which confirms the importance of fertilization with this nutrient. The response to sulfur fertilization depends on the content of this nutrient in the soil and the content of organic matter or fertilization nitrogen [18]. For this reason, studies have shown that the absence of sulfur does not affect the development of grasses [1,6]. Therefore, in view of the scarcity of sulfur in the soil, sulfur fertilization impacts the productivity of all cultivars of *Urochloa brizantha* so that Piatã is the cultivar with the greatest restriction due to the absence of sulfur, although the occurrence of chlorosis has not been observed (Table 3).

The absence of chlorosis observed in this experiment, despite sulfur deficiency, may be related to the duration or severity of the deficiency not being sufficient to cause visible pigment loss. Additionally, some forage species can maintain green leaves (without yellowing) under moderate sulfur limitation by reallocating internal reserves or through metabolic adjustments. It has been reported that sulfate deficiency reduces the synthesis of the Rubisco enzyme (ribulose-1,5-bisphosphate carboxylase/oxygenase), decreasing CO_2 assimilation rates and slowing carbohydrate synthesis, which often results in chlorosis in young leaves [5]. However, physiological impairments, such as reduced growth, can occur before the appearance of visible symptoms like chlorosis, which explains the un-changed chlorophyll index despite reductions in plant development.

Further studies should compare the productive effect of the absence of sulfur in an economic evaluation, since sulfur fertilization increases the cost of production. In view of this, the increase in cost may be more limiting than the productive restriction of the absence of this nutrient. In addition, further research should highlight the potential for the recovery of cultivars without sulfur from maintenance fertilization strategies.

5. Conclusions

Sulfur deficiency limits the establishment of *Urochloa brizantha*. Among the evaluated cultivars, Marandu is the least affected by sulfur deficiency during this stage, whereas Piatã is the most affected, demonstrating differences in nutritional requirements within the species. This variability highlights the importance of adopting cultivar-specific management strategies to optimize production and improve nutrient use efficiency.

Future research should be conducted under field conditions and in soils with varying sulfur availability to better understand the consistency of these responses. Moreover, the use of alternative sulfur sources, such as gypsum and elemental sulfur, during pasture establishment should be evaluated as potential strategies to enhance the productivity of forage systems.

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