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Article

Inoculation of plant growth-promoting bacteria and silicon to optimize nitrogen fertilization in Emerald grass

Uso de bactérias promotoras de crescimento e silício para otimização da adubação nitrogenada em grama esmeralda

Vitória Almeida Moreira Girardi[1,*], Carlos Eduardo da Silva Oliveira[2], Bruno Horschut de Lima[1], Isabela Martins Bueno Gato[1], Patrick Luan Ferreira dos Santos[3], Nelson Câmara de Souza Júnior[1], Arshad Jalal[1] and Marcelo Carvalho Minhoto Teixeira Filho[1]

[1] Universidade Estadual Paulista, Faculdade de Engenharia (FEIS), Departamento de Fitossanidade, Engenharia Rural e Solos, Ilha Solteira-SP, Brasil.

[2] Universidade Estadual de Mato Grosso do Sul, Departamento de Agronomia, Cassilândia-MS, Brasil

[3] Universidade Estadual Paulista, Faculdade de Ciências Agronômicas (FCA), Departamento de Ciência Florestal, Solos e Ambiente, Botucatu-SP, Brasil.

Abstract: In Brazil, the main species of turfgrass produced and sold is Emerald grass (*Zoysia japonica* Steud). However, for high quality production an intensive program of nitrogen fertilization and irrigation is necessary, representing around 21.5% of the costs. Nitrogen is crucial for plant nutrition and growth, increasing photosynthesis and promoting an intense green color. A sustainable alternative is the use of plant growth-promoting bacteria (PGPB), such as *Pseudomonas fluorescens* and *Azospirillum brasilense*, which reduce costs and increase profitability by promoting plant growth. Silicon (Si) fertilization also improves plant resistance to biotic and abiotic stresses. The objective of this study was to evaluate the performance of Emerald grass under the effect of inoculation with BPCPs combined with the supply of Si and doses of N, aiming to reduce nitrogen fertilization by 25%. The experiment evaluated soil cover, dark green color index, vegetation index by normalized difference and mass of Emerald grass sod, with 12 treatments combining different doses of N, application of Si and inoculation with BPCPs. The results indicated that the recommended dose of N promoted a higher rate of soil coverage, as well as providing a more intense green in *Z. japonica*, however, 75% of the recommended dose of N caused greater mass of the mats. However, there was no significant effect of inoculation with BPCPs, nor of Si supply on the analyzed variables.
Keywords: *Azospirillum brasilense*, N supply, *Pseudomonas fluorescens*, Si supply, *Zoysia japônica.*

Resumo: No Brasil, a principal espécie de grama produzida e comercializada é a grama Esmeralda (*Zoysia japonica* Steud). Todavia, para a produção de alta qualidade é necessário um programa intensivo de adubação nitrogenada e irrigação, representando cerca de 21,5% dos custos. O nitrogênio é crucial para a nutrição e crescimento das plantas, aumentando a fotossíntese e promovendo uma coloração verde intensa. Uma alternativa sustentável é o uso de bactérias promotoras de crescimento de plantas (BPCPs), como *Pseudomonas fluorescens* e *Azospirillum brasilense*, que reduzem custos e aumentam a lucratividade, promovendo o crescimento das plantas. A adubação com silício (Si) também melhora a resistência das plantas a estresses bióticos e abióticos. Objetivou-se com este estudo avaliar o desempenho da grama Esmeralda sob o efeito da inoculação com BPCPs aliado ao fornecimento de Si e doses de N, visando à redução em 25% da adubação nitrogenada. O experimento avaliou a cobertura do solo, índice de cor verde escuro, índice de vegetação por diferença normalizada e massa dos tapetes de grama Esmeralda, com 12 tratamentos combinando diferentes doses de N, aplicação de Si e inoculação com BPCPs. Os resultados indicaram que a dose recomendada de N promoveu maior taxa de cobertura do solo, assim como proporcionou um verde mais intenso em *Z. japonica*, todavia, a aplicação de 75% da dose recomendada de N ocasionou maior massa dos tapetes. Contudo, não houve efeito significativo da inoculação com as BPCPs, nem do fornecimento de Si nas variáveis analisadas.
Palavras-chave: *Azospirillum brasilense*, fornecimento de Si, *Pseudomonas fluorescens*, suprimento de N, *Zoysia japônica.*

Introduction

In Brazil, the production of cultivated turfgrass was first recorded in 1966 in the municipality of Curitiba, State of Paraná (Villas Bôas et al., 2020), where it is currently estimated that the sod production area has an average occupation of 32.5 thousand hectares in the country, and around 79% of these cultivated turfgrass species are Zoysias (Santos and Carribeiro, 2022). Thus, Emerald grass (*Zoysia japonica* Steud) is the most commercialized species and used in the formation of urban and residential gardens, sports areas, industrial parks, public works, highways, and slope containment, demonstrating good adaptation to different locations (Gazola et al., 2019; Castilho et al., 2020).

To achieve adequate levels of high-quality sod production, it is necessary to implement an intensive nitrogen fertilizer program with high doses, together with irrigation, which results in significant expenses for producers. These expenses represent approximately 21.5% of the total sod production costs (Agrianual, 2023).

Nitrogen influences several important characteristics in turfgrass management (Mateus et al., 2020; Santos et al., 2022), considered the nutrient used by plants in the greatest quantity, directly affecting crop yield, as it plays important roles in nutrition and plant growth (Godoy et al., 2012; Omara et al., 2020). This nutrient is essential for maintaining the aesthetic quality of lawns, promoting an intense green color (Santos et al., 2019), in addition, plants with an adequate N content, physiologically, have a greater photosynthetic capacity to synthesize carbohydrates given the higher concentration of chlorophyll, promoting better capture of light energy from solar radiation (Godoy et al., 2017; Gazola et al., 2019; Prates et al., 2020).

In this context, one of the alternatives for reducing nitrogen fertilization is the use of plant growth-promoting bacteria (PGPBs), such as *Pseudomonas fluorescens* and *Azospirillum brasilense*, which can provide a sustainable form of production, with increased profitability for rural producers as bacteria are a low-cost technology compared to fertilizer. And due to the success achieved, research on inoculation with PGPBs has grown rapidly, not only in grasses and legumes, but also in forestry, ornamental, fruit and vegetable species (Teixeira Filho and Galindo, 2019).

Studies were carried out with the inoculation of *A. brasilense* in grasses, such as corn (Souza et al., 2019), wheat (Galindo et al., 2020) and turfgrass (Santos et al., 2024). Furthermore, Zhang et al. (2018) highlight that the combined application of *A. brasilense* and *P. fluorescens* in the rice rhizosphere enhanced nitrogen conversion and availability in the soil. Additionally, co-inoculation led to increased crop biomass, with more significant effects observed when both bacteria were applied together.

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Another practice that has numerous benefits on grasses has been fertilization with silicon (Si). This is the second most abundant element in the Earth's crust, right after oxygen (Neu et al., 2017). However, the availability of Si in tropical soils is restricted, due to high levels of weathering and ferralization of primary silicate minerals (Linden et al., 2019). According to Etesami (2018), the use of Si combined with PGPBs can be an interesting and sustainable strategy to improve plant development in suboptimal conditions. Fertilization with Si promotes beneficial effects mainly when plants are subjected to biotic and abiotic stresses, common in adverse soil and climate conditions. This element, when added to the soil, or via foliar or even nutrient solution, provides beneficial effects for the plant, making it more tolerant to possible attacks by pests and pathogens, reducing water loss through evapotranspiration, promoting better use humidity (Godoy et al., 2012). However, there are no studies related to the strategic use of silicon combined with inoculation with PGPBs in Emerald grass, which is a market to be explored.

In view of the above, the objective of this study was to evaluate the use of growth-promoting bacteria (*P. fluorescens* and *A. brasilense*) combined with the supply of silicon and doses of N, aiming at a 25% reduction in nitrogen fertilization, greater mass of the mats, as well as the quality of sod production.

Material and methods

Location, management history and climatic characteristics of the experimental area

The study was carried out between October 2021 and August 2022 in an area of Emerald grass production, at an average altitude of 343 m, with sandy-textured soil classified as dystroferric Red Latosol (Santos et al., 2018) in the Brazilian soil taxonomy. The minimum and maximum monthly temperatures (ºC) were obtained from a meteorological station via a climate channel, and the monthly precipitation (mm) was collected on site (Fig. 1).

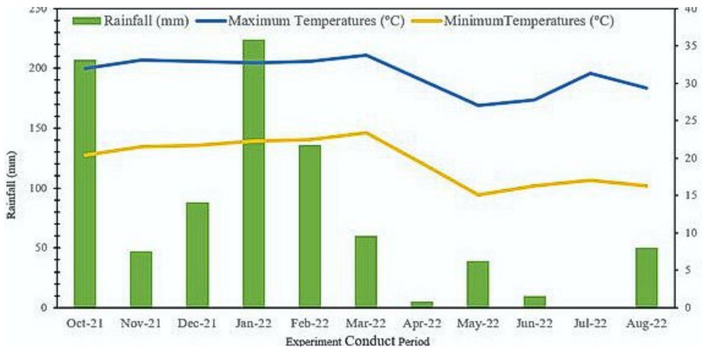

Fig. 1. Minimum and maximum temperatures and rainfall during the experiment with Emerald grass.

The sod production area for this experiment covers 117 hectares, with turfgrass cultivation irrigated through a central pivot system, providing around 10 mm of water daily from 9:40 pm to 6:00 am in the absence of rain. Emerald grass has a rhizomatous and stoloniferous growth habit, allowing it to be harvested across the entire area, as the subsurface rhizomes sprout and recover the soil after harvest. The cultivation period varies from 8 to 12 months, and the experimental area has been cultivated with this species for the past 20 years.

Experimental design and treatments

The experiment was carried out using a randomized block design with 12 treatments arranged in a 3 x 2 x 2 factorial scheme, with four replications, in plots of 9 m². The treatments consisted of two inoculations with PGPB (*Azospirillum brasilense* and *Pseudomonas fluorescens*), along with a control (no inoculation), combined with two doses of N: 100% of the routine dose applied by the producer company (280 kg N ha^{-1} $year^{-1}$) and 75% of the dose (210 kg N ha^{-1} $year^{-1}$), divided into four installments. The experiment also included treatments with or without silicon supply via fertilization with potassium, using either a commercial fertilizer (12% K_2O and 25% total Si) or Potassium Chloride (60% K_2O), applied in doses of 240 kg ha^{-1} of K_2O, divided into four applications.

Characterization and Experimental Conduct

The plots were delimited on October 22, 2021, after harvesting the sod from the previous cycle. At this time, soil samples were collected at a depth of 0.00 - 0.20 m to analyze the chemical attributes of the soil (Table 1), following to the methodology of Raij et al. (2001).

Table 1. Chemical attributes of the soil in the 0.00 to 0.20 m layer before the installation of the Emerald grass experiment.

	$P_{(resin)}$	OM	pH	K	Ca	Mg	H+Al	SB	CEC
	(mg dm^{-3})	(g dm^{-3})	($CaCl_2$)	------------------- $mmol_c$ dm^{-3} -------------------					
	36.00	12.00	5.00	2.00	11.00	4.00	15.00	17.00	32.00
V	**m**	**Al**	**$S\text{-}SO_4^{2-}$**	**B[a]**	**Cu[b]**	**Fe[b]**	**Mn[b]**	**Zn[b]**	**Si**
%	%	$mmol_c$ dm^{-3}	mg dm^{-3}	--------------- mg dm^{-3}---------------------					
53.0	0.00	0.00	7.00	0.07	0.60	39.00	12.40	0.10	4.00

Note: [a]Determined in hot water, [b]Determined in DTPA (diethylenetriaminepentaacetic acid). OM: Organic Matter. CEC: cation exchange capacity, SB: sum of bases, V: base saturation, m: Al saturation.

The phosphate fertilizer with triple superphosphate (444.00 kg ha^{-1}, 45% P_2O_5) was the same for all treatments, applied 199 days after harvesting the previous sod and 178 days after the application of growth-promoting bacteria. Soil decompression and liming (1,000 kg ha^{-1}, 80% Relative Total Neutralization Power - RTNP) were carried out on October 22, 2021, after harvesting and sampling the ground. The dosage of P_2O_5 and the supply of limestone are necessary due to the removal of a layer of soil during the sod harvest, in addition to being an acidic tropical soil, highly compacted due to the management practices adopted in the production area, with high phosphorus adsorption. The use of incorporators is not employed, and, as a result, there is no incorporation of fertilizers and amendments applied to the subsoil. Therefore, the applied dosage of P_2O_5 and limestone is necessary to meet the demand for P, Ca, and Mg in the system for a production cycle. Turfgrass management was carried out in accordance with the sod producing company's routine, including all chemical control of weeds and pests, in addition to mowing and removing clippings.

The application of PGPBs was carried out 21 days after harvesting the previous sod, on November 12, 2021, with 200 mL ha^{-1} of the inoculant (manufacturer's recommendation) containing PGPBs diluted in a solution of 400 L ha^{-1} and sprayed with a backpack pump on damp ground. At the time of inoculation, the area had approximately 5% soil coverage by the vegetative part of the grass. The purpose of this application was to reach the soil so that the PGPB could act specifically in the rhizosphere and it was applied across the entire soil surface of the designated area in each plot. Inoculations were performed once, between 8 and 9 am. Liquid bacterial inoculants of *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) and *Pseudomonas fluorescens* (strain CCTB03) contain a guarantee of 2×10^8 mL^{-1} Colony Forming Units (CFU).

The doses of N, using urea as a source (45% N), fertilization with Si (with the application of the commercial product) and the non-supply of this element (with the use of fertilizer based on potassium chloride), were applied manually, always in the afternoon, with nighttime irrigation and repeated every two months (totaling four applications) until the soil was completely covered by turfgrass. The first fertilization carried out on 11/17/2021, the second on 01/10/2022 (59 DAA - Days After Application), the third on 03/10/2022 (118 DAA), and the fourth on 05/09/2022 (178 DAA).

Evaluations carried out

a) Coverage Rate (CR): Carried out on 01/10/2022 (59 DAA), 02/10/2022 (90 DAA), and 04/11/2022 (143 DAA) by the analysis of the digital image. In this research, digital images were obtained from a digital camera, 12 megapixels and a standard height of 1.3 m, so that the images were recorded parallel to the lawn surface, always at the same height, avoiding shadows of the photographer or any part of the camera. Each figure was downloaded and analyzed using specific software for measuring green coverage.

b) Intensity of turfgrass green color: Carried out on 01/10/2022 (59 DAA), 02/10/2022 (90 DAA), and 04/11/2022 (143 DAA) also by analyzing the shoot digital image, using the methodology present in the work of Godoy et al. (2012). The captured images were transferred and analyzed using computational software, the RGB (Blue, Green and Red) value of each image was determined. As only the green component (G) does not define the green color, also depending on the red (R) and blue (B) components, the RGB results were compiled into a spreadsheet and converted to HSB (Hue, Saturation and Brightness) values, and with these, the Dark Green Color Index (DGCI) was calculated, which ranges from 0 – 1 (Karcher and Richardson, 2003).

c) Normalized Difference Vegetation Index (NDVI): Carried out on 01/10/2022 (59 DAA), 02/10/2022 (90 DAA), and 04/11/2022 (143 DAA), using portable equipment, positioned parallel to the grass surface at a height of 1.3 m, with three readings being taken on each plot to calculate the average (Nascimento et al., 2020, Silva et al., 2020).

d) Sod mass: Carried out on 08/04/2022 (265 DAA – approximately eight months after sod harvesting from the previous cycle and installing treatments), after irrigation and the passage of the compactor roller, both in the total area, they were weighed, on an analytical scale, using three sod (with a soil thickness of approximately 3 cm) to measure the average weight in kg for each plot.

Statistical analysis

The data were subjected to an analysis of variance using the F-test with a probability of 5% and 1%. The significance of the mean squares obtained in the analysis of variance was tested with the Tukey test ($p \leq 0.05$) using the calculation software (Ferreira, 2019).

Results and Discussion

Coverage Rate (CR) and Sod Mass

There was a significant effect ($p \leq 0.01$) of N doses at 90 and 143 DAA for CR in which the 100% N dose provided an increase of 10.0% and 12.0% in CR at 90 and 143 DAA, respectively, compared to the supply of 75% of the N dose (Table 2). The emphasis is on the importance of fertilization in the turfgrass development and the consequent covering of the soil to form sod. Work by Lima et al. (2015) and Mota et al. (2019) presented similar results, that is, the higher the dose of N applied, the faster and greater the closure of sod and, consequently, the soil coverage rate.

Significance of inoculations on CR was found at 143 DAA ($p < 0.01$) with higher CR (10.6% and 8.8%) in the control compared to inoculation with *A. brasilense* and *P. fluorecens*, respectively, at 143 DAA (Table 2). Research such as those by Dias et al. (2018), Santos et al. (2019) and Amaral et al. (2019), confirm that the use of organic substrates and greater input of organic matter are essential for the good development of turfgrass species, which can also help inoculation and increase microbial flora.

The soil in question for conducting this experiment has only 1.2% organic matter, a considerably low level due to the cuts made to sod harvest. This may be one of the factors why bacteria did not promote significant statistical differences for this study. However, the microbial community can also negatively impact plant productivity by competing for nutrients or contributing to their loss in the soil through the formation of mobile forms prone to leaching (Matos et al., 2016). This phenomenon may have influenced the low soil coverage by grass in the present study.

A significant effect ($p \leq 0.05$) was noted for N doses (Table 2). The lowest dose of nitrogen provided a 9.4% increase in the mass of the sod compared to the highest dose (Table 5); therefore, it is inferred that the number of rhizomes for new growth would probably be greater in the next cycle. In a study conducted by Backes et al. (2013), with Empire turfgrass (*Zoysia japonica*), values between 4 and 4.75 kg were obtained for newly harvested sod, which are lower than the results found in this work. The authors highlight that this characteristic has practical implications, as a smaller mass allows a greater number of sods to be transported with the same load.

Prates et al. (2020), working with Bermudagrass and nitrogen doses in winter, also found that higher doses of the nutrient are more responsive in terms of production and green color balance. The authors highlight that to produce good sod, it is essential to combine the commercial/economic aspect with responses in the field of cultivation. Therefore, the ideal dose for maximum productivity may differ from the ideal economic dose feasible for nitrogen supply, making field and economic research essential to define the best dose. Santos et al. (2020), working with Emerald grass, states that sensory factors must also be taken into consideration when defining the best nitrogen fertilizer to use in turfgrass management.

Table 2. Soil Coverage Rate (CR) by Emerald grass at 59, 90, and 143 days after application (DAA) of plant growth-promoting bacteria (PGPBs) and sod mass after harvest.

F.V.	CR			Sod mass
		%		kg
	59DAA	90DAA	143DAA	-
Block	0.05	0.04	0.05	0.75 ns
Supply of Si (S)	0.54 ns	0.52 ns	0.82 ns	0.53 ns
Dose of N (%) (D)	0.13 ns	0.00**	0.00**	0.04*
Inoculation (I)	0.17 ns	0.25 ns	0.00**	0.26 ns
CV (%)	23.94	9.64	8.41	13.49
Overall Average	44.32	63.28	74.30	6.76
MSD	6.23	3.58	3.67	0.53
Supply of Si				
Without	45.28	64.00	74.10	6.67
With	43.37	63.00	74.50	6.84
Dose of N (%)				
75	41.93	60.00 b	70.00 b	7.00 a
100	46.72	66.00 a	78.70 a	6.49 b
Inoculation				
No inoculation	46.72	64.90	79.00 a	6.60
A. brasilense	46.11	63.75	71.39 b	6.60
P. fluorescens	40.15	61.27	72.61 b	7.07

ns - Not significant by F test; *, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively. Averages followed by different letters in the column differ using the Tukey test at 5%. 100% and 75% of the N dose is equivalent to 280 and 210 kg ha^{-1} of N (respectively) divided into 4 applications. MSD: minimum significant difference.

Dark Green Color Index (DGCI) and Normalized Difference Vegetation Index (NDVI)

A significant effect of N doses on DGCI ($p \leq 0.01$) (Table 3) was found at 90 DAA and 143 DAA, in which the supply of the highest dose of N provided a greater DGCI of the turfgrass at these two times analyzed. A well-nourished turfgrass tends to display an intense green color, if it is not subject to other types of stress, whether biotic or abiotic. Mota et al. (2019), studying with doses of sewage sludge, found that after 212 days, organic fertilizer that provided 300 kg ha^{-1} of nitrogen was able to increase production, with sod occupying 100% of the soil cover, in addition to an increase in dark green color, brightness and rapid closure of gaps.

Therefore, the increase in the green tone of the leaves, associated with the higher DGCI due to the application of higher doses of nitrogen, may be related to the presence of greater amounts of chlorophyll a and b in the leaves of Emerald grass. Santos et al. (2019), in their research with Bermudagrass, also found that a greater supply of nitrogen was able to increase the levels of photosynthetic pigments and the nitrogen content in the leaves, which considerably increased the green color intensity.

Table 3. Dark Green Color Index (DGCI) and Normalized Difference Vegetation Index (NDVI) of Emerald grass at 59, 90, and 143 days after application (DAA) of plant growth-promoting bacteria (PGPB).

F.V.	DGCI			NDVI		
	59DAA	90DAA	143DAA	59DAA	90DAA	143DAA
Block	0.00	0.28	0.22	0.00	0.17	0.60
Supply of Si (S)	0.82 ^{ns}	0.99 ^{ns}	0.40 ^{ns}	0.97 ^{ns}	0.61 ^{ns}	0.28 ^{ns}
Dose of N (%) (D)	0.21 ^{ns}	0.00**	0.01**	0.81 ^{ns}	0.00**	0.00**
Inoculation (I)	0.45 ^{ns}	0.13 ^{ns}	0.38 ^{ns}	0.22 ^{ns}	0.50 ^{ns}	0.19 ^{ns}
CV (%)	18.09	18.32	18.53	16.56	7.10	5.83
Overall Average	0.45	0.37	0.39	0.50	0.59	0.61
MSD	0.04	0.04	0.04	0.04	0.02	0.02
Supply of Si						
Without	0.45	0.38	0.40	0.50	0.59	0.60
With	0.45	0.38	0.38	0.50	0.59	0.62
Dose of N (%)						
75	0.44	0.34b	0.37b	0.50	0.57b	0.60b
100	0.47	0.41a	0.42a	0.51	0.61a	0.63a
Inoculation						
No inoculation	0.44	0.40	0.41	0.53	0.60	0.62
A. brasilense	0.44	0.35	0.38	0.50	0.58	0.61
P. fluorescens	0.48	0.38	0.38	0.48	0.59	0.60

ns - Not significant by F test; *, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively. Averages followed by different letters in the column differ using the Tukey test at 5%. 100% and 75% of the N dose is equivalent to 280 and 210 kg ha^{-1} of N (respectively) divided into 4 applications. MSD: minimum significant difference.

Silva et al. (2020) also observed an increase in DGCI with increasing doses of N, applied organically to Bermudagrass. However, the supply of Si or not and the inoculation with PGPB did not affect DGCI at any of the times analyzed.

There were significant values ($p \leq 0.01$) for the N doses on NDVI at 90 and 143 DAA, in which the 100% N dose provided an increase of 7% and 5%, respectively, compared to the 75% N dose (Table 3). Nitrogen plays a direct role in the chlorophyll molecule and, as a result, directly affects NDVI, which is related to the amount of chlorophyll and energy absorption. On the other hand, there was no significant effect for inoculation and the supply or not of Si at any of the times analyzed for NDVI.

The portable NDVI sensor helps to identify the spatial variability of biomass production and operates as an indicator of the need for fertilizer to be applied for the crop in question, contributing to the efficiency in the use of fertilizers, especially nitrogen fertilizers (Nascimento et al., 2020; Silva et al., 2020; Santos et al., 2024).

Marín et al. (2020), reported that NDVI correlates with the turfgrass quality, and can be used as an additional instrument in irrigation management, considering that the higher the vegetation index, the better the condition of the turfgrass. However, values below ideal may indicate water deficit, lack of nutrients or diseases, therefore, before adjusting irrigation, other factors that also affect the quality of the lawn must be discarded.

Gazola et al. (2019), in a research with Emerald grass, glyphosate and doses of nitrogen, make it clear that lower doses of nitrogen fertilizers can reduce the lawn's coverage rate and increase yellowing. The authors state that nitrogen distribution is essential to promote visual quality (important for market), based on data found by the chlorophyll index in the leaves. In this way, the NDVI test can be an aggregator for turfgrass management, to better understand the chlorophyll balance in the plant.

Therefore, under the experimental conditions evaluated, inoculation with PGPBs as well as the application of Si did not contribute to the improvement of turfgrass quality parameters or to the efficient reduction of nitrogen use. Therefore, future research is necessary to explore other factors that may enhance the effects of these treatments, including different soil conditions, climate and management practices, as well as the evaluation of other biostimulants and application doses.

Conclusions

The recommended dose of N promoted a higher rate of soil coverage, as well as providing a more intense green in *Z. japonica*, however, 75% of the recommended dose of N caused greater sod mass. However, there was no significant effect of inoculation with PGPBs (*P. fluorescens* and *A. brasilense*) and Si supply on the analyzed variables.

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Author Contribution

VAMG: Research, Methodology, Data Curation, Formal Analysis, Writing - Original Draft & Writing - Review & Editing. **CESO:** Research, Data Curation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing. **BHL:** Research, Data Curation, Formal Analysis**. IMBG:** Research, Data Curation, Formal Analysis. **PLFS:** Conceptualization, Formal Analysis, Writing – Review & Editing. **NCSJ:** Conceptualization, Formal Analysis, Writing – Review & Editing. **AJ:** Conceptualization, Formal Analysis, Writing – Review & Editing. **MCMTF:** Conceptualization, Project Administration, Resources, Supervision, Writing – Review & Editing.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors**.**

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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