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Microbial Inoculants and Fertilizer Reduction in Sorghum Cultivation: Implications for Sustainable Agriculture

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Abstract: Sorghum (*Sorghum bicolor* L. Moench) is a versatile cereal crop with diverse applications in human food, animal feed, and other industries. This study investigated the effects of microbial inoculation on sorghum growth and nutrient uptake at two fertilizer levels (100% and 80% of the recommended dose). *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *Purpureocillium lilacinum*, and *Trichoderma harzianum* were applied to the soil and plants in a greenhouse experiment using a completely randomized design with six replicates per treatment. Plant growth parameters, including height, shoot and root dry matter, nitrogen and phosphorus content in the shoots and roots and chlorophyll, were assessed. The results showed no statistically significant differences among the treatments for most parameters, except for plant height and shoot dry matter, where the *B. subtilis* treatment exhibited the lowest values. Notably, treatments that received 80% of the recommended fertilizer dose performed similarly to those that received 100%, suggesting the potential for reduced fertilizer usage with microbial inoculants. Although the microbial treatments did not significantly enhance sorghum growth in this study, evaluating their effects remains crucial for developing eco-friendly alternatives to reduce chemical fertilizers. Further research is needed to optimize the application of microbial inoculants and to understand their impact on soil health and agricultural productivity under various environmental conditions.

Keywords: plant growth-promoting; plant production; sustainable production; environmental impact reduction



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1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal crops and ranks as the fifth most significant grain crop worldwide [1]. It serves as a staple food source for over 300 million people in Africa and Asia, and is cultivated for various purposes, including human food, animal feed, renewable energy, and industrial applications [2].

The importance of sorghum extends beyond its traditional use. It is increasingly recognized as a promising feedstock for the production of bioactive compounds, particularly kafirin protein fraction, which can generate biologically active peptides with antioxidant, anticancer, antimicrobial, and anti-inflammatory properties [1]. In addition, sorghum is gaining popularity as a gluten-free alternative to wheat, making it suitable for patients with celiac disease [3].

The global importance of sorghum stems from its versatility, nutritional value, and adaptability to harsh environmental conditions. Its ability to produce reasonable yields in

poor soil and limited rainfall conditions makes it a crucial crop for food security in arid and semi-arid regions [4]. Furthermore, the potential of sorghum as a second-generation biofuel crop, offering ethanol from grains, stems, and biomass, is significant for addressing global energy challenges [5].

Sorghum is gaining importance as a valuable feed grain for livestock, particularly in regions that face challenging environmental conditions. Its versatility and nutritional properties make it an attractive option for animal feed production. Sorghum can be utilized by both non-ruminant and ruminant production systems as a source of energy and protein [4]. Sorghum can serve as the primary grain source in animal diets when processed correctly, and in balance with other feed ingredients. The feeding value of sorghum for livestock is generally 95% or more of that of yellow dent maize [6]. This makes it a suitable alternative to corn for animal feed preparation.

Interestingly, although early studies have indicated that sorghum-based diets are inferior to corn-based diets in terms of animal performance, recent studies have shown no significant difference between the two. This improvement was attributed to the development of low-tannin sorghum varieties [7]. Additionally, sorghum has a lower incidence of mycotoxins than corn, further enhancing its suitability as animal feed.

The drought tolerance, adaptability to various environmental conditions, and improved nutritional profile of sorghum make it an important crop for animal feed. Its potential to meet the growing demand for high-quality feed at affordable prices, especially in developing countries, highlights its significance in the livestock industry [8]. As research continues to enhance sorghum grain quality and nutritional value, the importance of sorghum in animal feed is likely to increase.

Sorghum production in various regions faces several challenges. Soil with low fertility requires high amounts of mineral fertilizers, which increases production costs and may cause environmental issues, including soil salinization, eutrophication, leaching, and the emission of greenhouse gases [9].

Plant growth-promoting microorganisms (PGPM), including bacteria and fungi, can significantly improve sorghum production through various mechanisms. PGPM, such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), enhance nutrient uptake, promote plant growth, and increase crop yield [10]. These microorganisms can improve sorghum biomass production by synthesizing hormones, fixing nitrogen, and solubilizing phosphate and potassium [10]. For instance, certain bacterial strains can increase grain starch content, whereas AMF can enhance the protein content in grains, leading to improved nutritional value of sorghum [11].

Concerning the application of *P. lilacinum* to sorghum crops, there is a lack of existing research on its use in this context. *P. lilacinum* has been studied in various commodities including pineapple, maize, soybean, and legumes [12]. It has shown potential as a biocontrol agent against plant-parasitic nematodes and as a plant growth promoter in crops, such as cotton, peanuts, and maize [13]. This fungus has also been evaluated for its effectiveness against root-knot nematodes in eggplants [14].

Interestingly, the combination of PGPR and AMF can have additive effects on grain composition, potentially fulfilling both consumer and industrial requirements [15]. Moreover, PGPM can help sorghum plants withstand biotic and abiotic stresses, reducing the need for agrochemicals and promoting sustainable agriculture [16].

Harnessing the potential of PGPM can lead to increased sorghum productivity while maintaining soil health. The use of these microorganisms as biofertilizers and biopesticides offers an eco-friendly approach to improve crop production [16]. However, it is important to note that the effectiveness of microbial inoculants can be limited by competition with indigenous strains and environmental factors, necessitating further research to optimize

their application in sorghum cultivation [17]. Although sorghum is important for both animal feed production and human consumption, limited information is available regarding the application of plant growth-promoting microorganisms (PGPM) in this crop.

The objective of this study was to verify whether inoculation with *B. subtilis*, *B. pumilus*, *B. licheniformis*, *P. lilacinum*, and *T. harzianum* could be tested under two soil fertility levels: 100% and 80% fertilization.

2. Materials and Methods

The microorganisms used in this study belonged to the collection of the Laboratory of Soil Microbiology, UNESP, Campus of Jaboticabal, Brazil. These microorganisms (bacteria and fungi), *B. subtilis* (accession number MZ133755), *B. pumilus* (accession number MZ133476), *B. licheniformis* (accession number MZ133757), *T. harzianum* (accession number MZ133758), *P. lilacinum* (accession number KY624227.1). These microorganisms were selected because of their growth-promoting characteristics such as phosphorus solubilization, biological nitrogen fixation, and IAA production [18–20].

For inoculum development, *B. subtilis*, *B. pumilus* and *B. licheniformis* were cultured in nutrient broth for 48 h at 28 °C, whereas *T. harzianum* and *P. lilacinum* were cultured in potato dextrose broth for 14 d at 28 °C. After the incubation period, the concentration was evaluated using the serial dilution method and standardized to 1×10^9 CFU mL⁻¹.

The microorganisms were subjected to two fertilizer conditions: the first utilizing 100% of the fertilizer, as determined by soil fertility analysis, and the second employing 80% of the fertilizer quantity, as indicated by soil fertility assessment. The treatments were as follows: T1, control treatment without microbial inoculation; T2, *B. subtilis*; T3, *B. pumilus*; T4, *B. licheniformis*; T5, *P. lilacinum*; and T6, *T. harzianum*.

The experimental setup was a completely randomized design within a greenhouse located in Jaboticabal, SP, Brazil (21°15'17" S, 48°19'20" W). The microorganisms were applied via the soil, and the inoculum at a concentration of 1×10^9 CFU mL⁻¹ was applied once directly to the soil in a volume of 10 mL per pot. During the 30-day study period, each treatment group received five weekly microbial reinoculations through foliar application, consisting of the same concentrations and volumes as the initial inoculation. Three sorghum plants were initially planted in each 5 L pot, which were subsequently thinned to two plants per pot after initial growth. The pots were filled up to 90% of their capacity with eutrophic red latosol soil, characterized by its chemical properties, including a pH of 6.9, 10% organic matter, 23 mg/dm³ available phosphorus, 0.7 mmolc/dm³ available potassium, 79 mmolc/dm³ calcium, 13 mmolc/dm³ magnesium, and 11 mmolc/dm³ hydrogen. Controlled environmental conditions were maintained in a greenhouse at a temperature of 24 ± 2 °C, $50 \pm 2\%$ relative humidity, and a light cycle of 16:8 h light to dark, thus fitting the region's Aw climate classification by Köppen and Geiger. In this study, sorghum plants were assessed using several growth parameters. The plant height was measured from the apex to the base of the plant. The biomass was then processed by splitting the shoots from the roots, which were dried in a forced ventilation oven at 65 °C for 72–96 h and subsequently weighed on a semi-analytical scale. The total dry mass of the plants was calculated by summing the weights of dried shoots and roots.

2.1. Determination of Nitrogen and Phosphorus in Shoots and Roots

Five hundred micrograms of dried and ground plant samples were weighed and placed into fifty mL digestion tubes, which were left to decouple at room temperature for 1.5 h. The tubes were then positioned in a digestion block and heated to 80 °C for 20 min before increasing the temperature to 160 °C. The tubes were monitored and removed once the material ascended the tube walls and most of the HNO₃ evaporated, leaving a clear

solution. After cooling, 1.3 the concentrated HClO_4 was added to each tube. The tubes were returned to the block, the temperature was increased to 210°C , and digestion was deemed complete when the solution became colorless with dense white vapors of HClO_4 and H_2O formed above the dissolved material. The tubes were cooled, and the contents were diluted to 25 mL with water in a snap-cap glass. For phosphorus analysis, 1 mL of the digested sample was transferred to a test tube to which 4 mL of water and 2 mL of reagent mix (comprising equal parts of 5% ammonium molybdate and 0.25% vanadate) were added. The mixture was allowed to rest for 15 min before measuring absorbance at 420 nm using a UVVIS spectrophotometer [21].

2.2. Chlorophyll Content

Chlorophyll content was measured using a spectrophotometer. Leaf squares were cut (1 cm^2) for this analysis. Each leaf square was then cut into smaller pieces and placed in an Eppendorf tube containing 5 mL of dimethylformamide. The tubes were stored at 8°C for 72 h in the dark. An aliquot of 3 mL of the liquid extract was then collected for reading in a spectrophotometer at 470, 647, and 664 nm. Chlorophyll content was determined using the following equation [22].

3. Statistical Analysis

Statistical analyses were conducted to evaluate the effects of different treatments on plant growth parameters and nitrogen and phosphorus content. Analysis of variance (ANOVA) was performed using an F-test in the AgroEstat program, version 1.1 [23]. Where significant differences were detected, mean comparisons were conducted using the Scott-Knott test at a 5% probability level. To assess the impact of each fertilizer dose, specifically 100% and 80%, on various parameters, ellipsis plots were analyzed for key parameters. Each subplot illustrates the relationship between two parameters, with ellipses representing the confidence region for each group.

4. Results

In terms of plant height, the lowest value was observed in T2, with no statistically significant differences detected among the other treatments. Similarly, no statistically significant differences were found among treatments with respect to plant diameter. Notably, there were no statistically significant differences in shoot dry matter (SDM) or root dry matter among the treatments (Figure 1).

Regarding root volume, no statistically significant differences were observed among treatments, and the highest P-SDM value was observed in treatment T2, which was the only treatment that significantly differed from the control and other treatments. For P-RDM, the lowest values were recorded in T7 and T12, with no significant differences among the other treatments. Finally, the highest values for P-Soil were found in treatments T7 to T11, compared to treatments T1 to T6, and T12 (Figure 2).

The highest N-Total values in the soil were observed in treatments T2, T3, and T10. In contrast, the other treatments exhibited lower values, which did not significantly differ from those of the control. The highest concentrations of chlorophyll a were observed in the T1, T2, T3, T7, T8, T9, T11, and T12 treatments. Conversely, the lowest concentrations were recorded in the T4, T5, T6, and T11 treatments. In the case of chlorophyll b, the highest concentrations were found in treatments T1, T2, T3, T7, T10, T11, and T12, whereas the lowest concentrations were noted in treatments T4, T5, and T6. The highest concentrations of chlorophyll a and b were observed in treatments T1, T2, T3, T7, T8, T9, T11, and T12. Conversely, the lowest concentrations were recorded in treatments T4, T5, T6, and T7 (Figure 3).

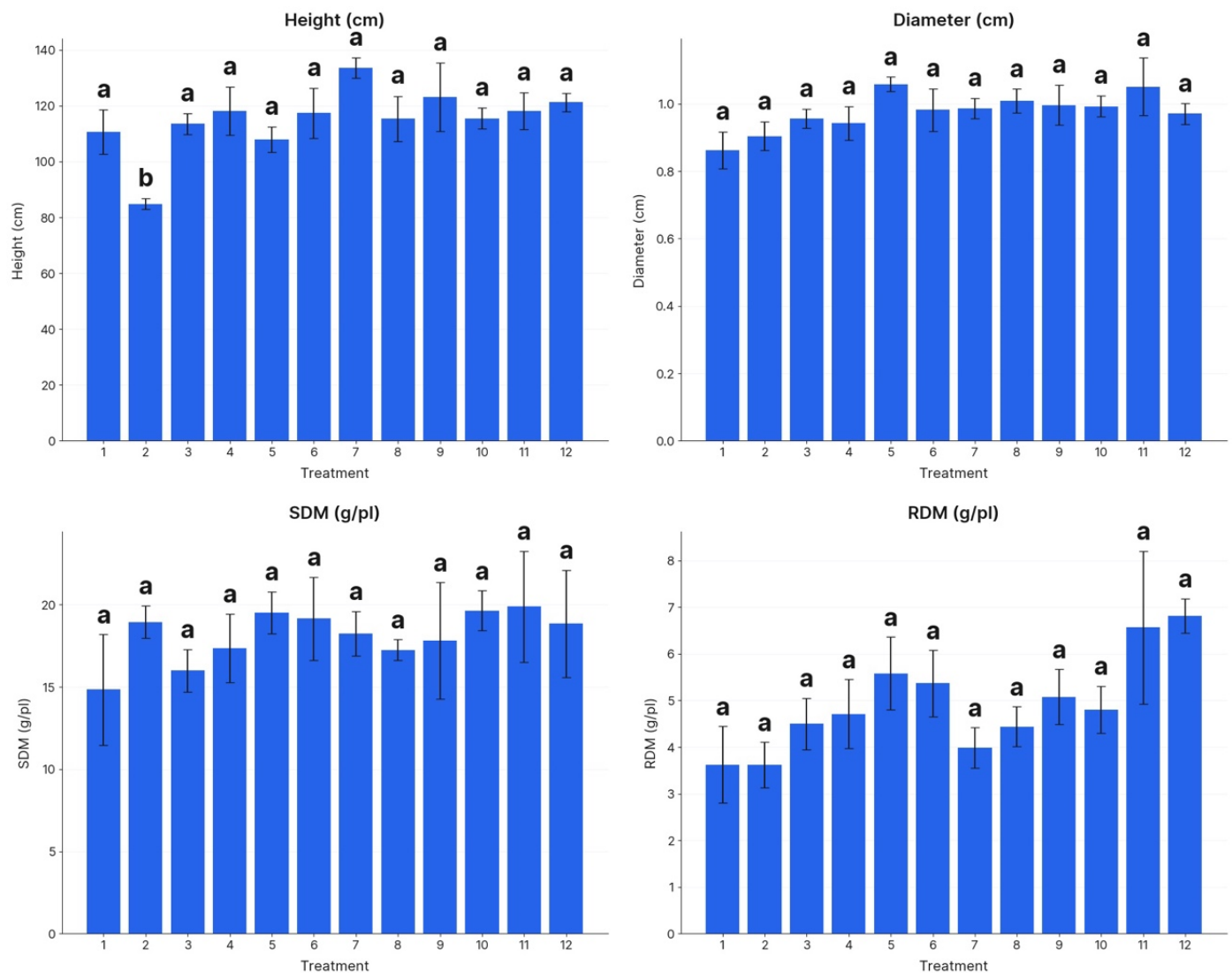


Figure 1. Mean values of the assessed parameters, including height (cm), diameter (cm), shoot dry matter (SDM) (g/plant), and root dry matter (RDM) (g/plant). Statistically significant differences among treatments are indicated by different letters.

Figure 4 presents ellipsis plots for key parameter pairs, categorized by fertilizer percentage (100% in blue, 80% in red). Each subplot illustrates the relationship between the two parameters, with ellipses delineating the confidence region for each fertilizer group. This visualization aids in understanding how varying fertilizer levels influence the distribution and correlation between plant growth and nutrient parameters.

Figure 5 shows the parameters most affected by fertilizer levels. The parameter P-Soil (mg kg^{-1}) was most dramatically affected, with a -57.3% difference between the 100% and 80% fertilizer levels. The soil phosphorus content was significantly higher in the 80% fertilizer treatment group. N-TOTAL (mg kg^{-1} soil) was the second most affected parameter, with a $+17.9\%$ increase in the 100% fertilizer treatment compared to the 80% treatment. Notably, the SDM (g/pl) shoot dry matter showed a -9.4% difference, with slightly higher values in the 80% treatment. Height (cm) exhibited a -8.2% difference, with taller plants in the 80% treatment. The least affected parameter was RDM (g/pl), root dry matter, showing a difference of -6.1% , and the diameter (cm) showed only a -5.0% difference. This suggests that soil nutrient parameters (P-Soil and N-TOTAL) were much more sensitive to fertilizer-level changes than morphological parameters (height, diameter, and biomass).

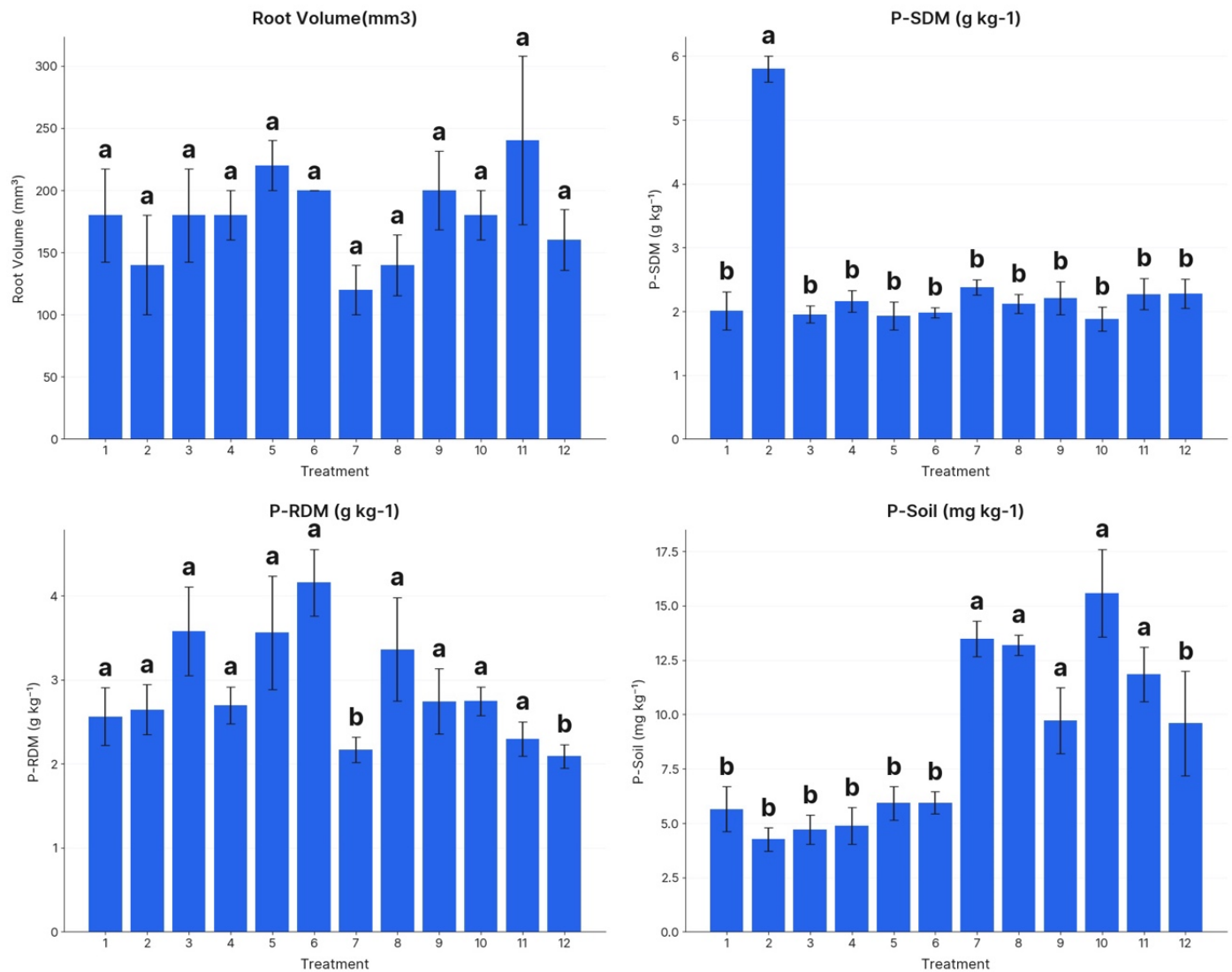


Figure 2. Average values of the analyzed parameters, including root volume (mm³), phosphorus content in shoot dry matter (P-SDM) (g/kg), phosphorus content in root dry matter (P-RDM) (g/kg), and phosphorus content in the soil (P-Soil) (mg/kg). Statistically significant differences among treatments are indicated by different letters.

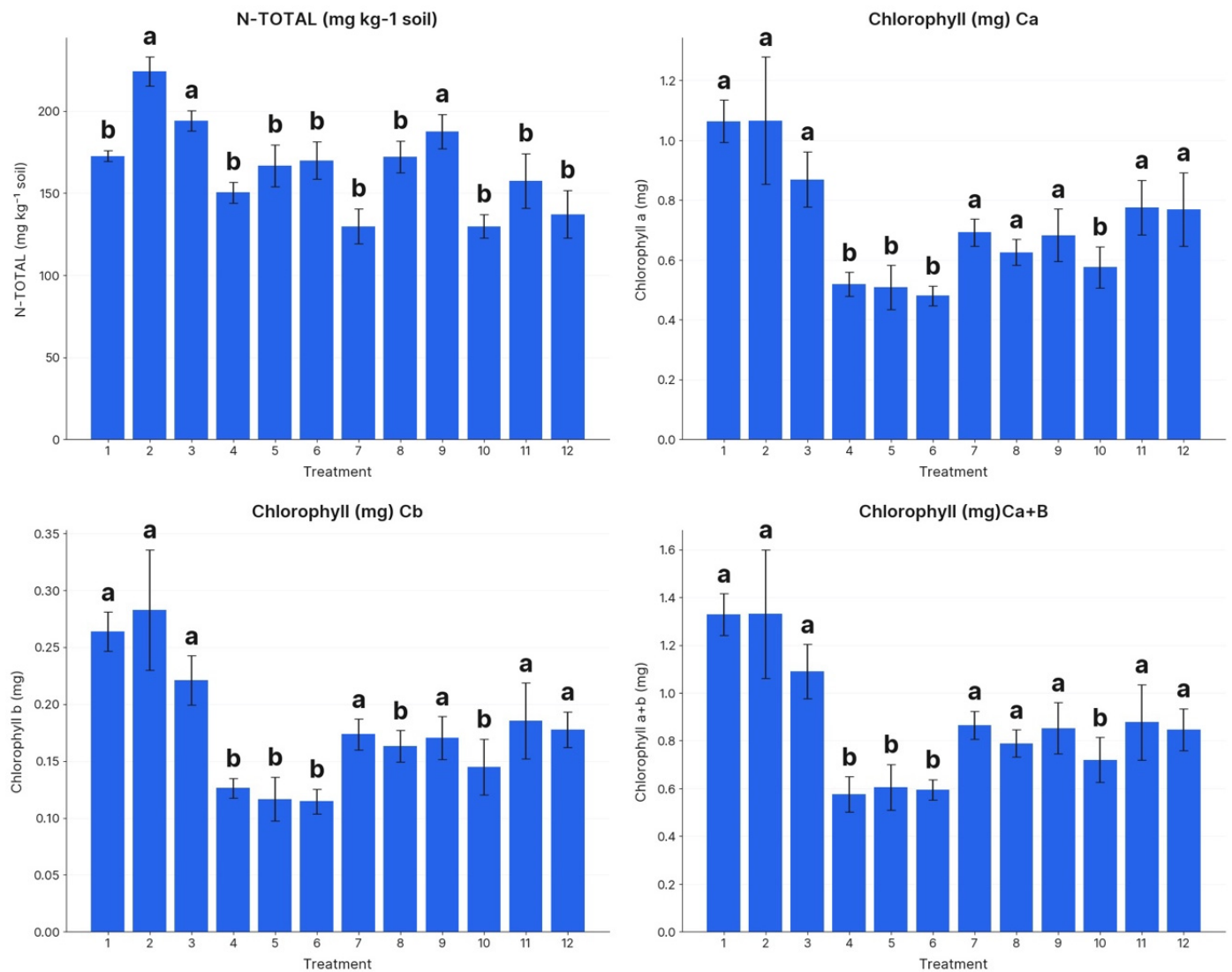


Figure 3. Average values of the analyzed parameters, including nitrogen total content (mg/kg of soil), chlorophyll (a) content (mg), chlorophyll content (b) (mg) and chlorophyll content (a + b) (mg). Statistically significant differences among treatments are indicated by different letters.

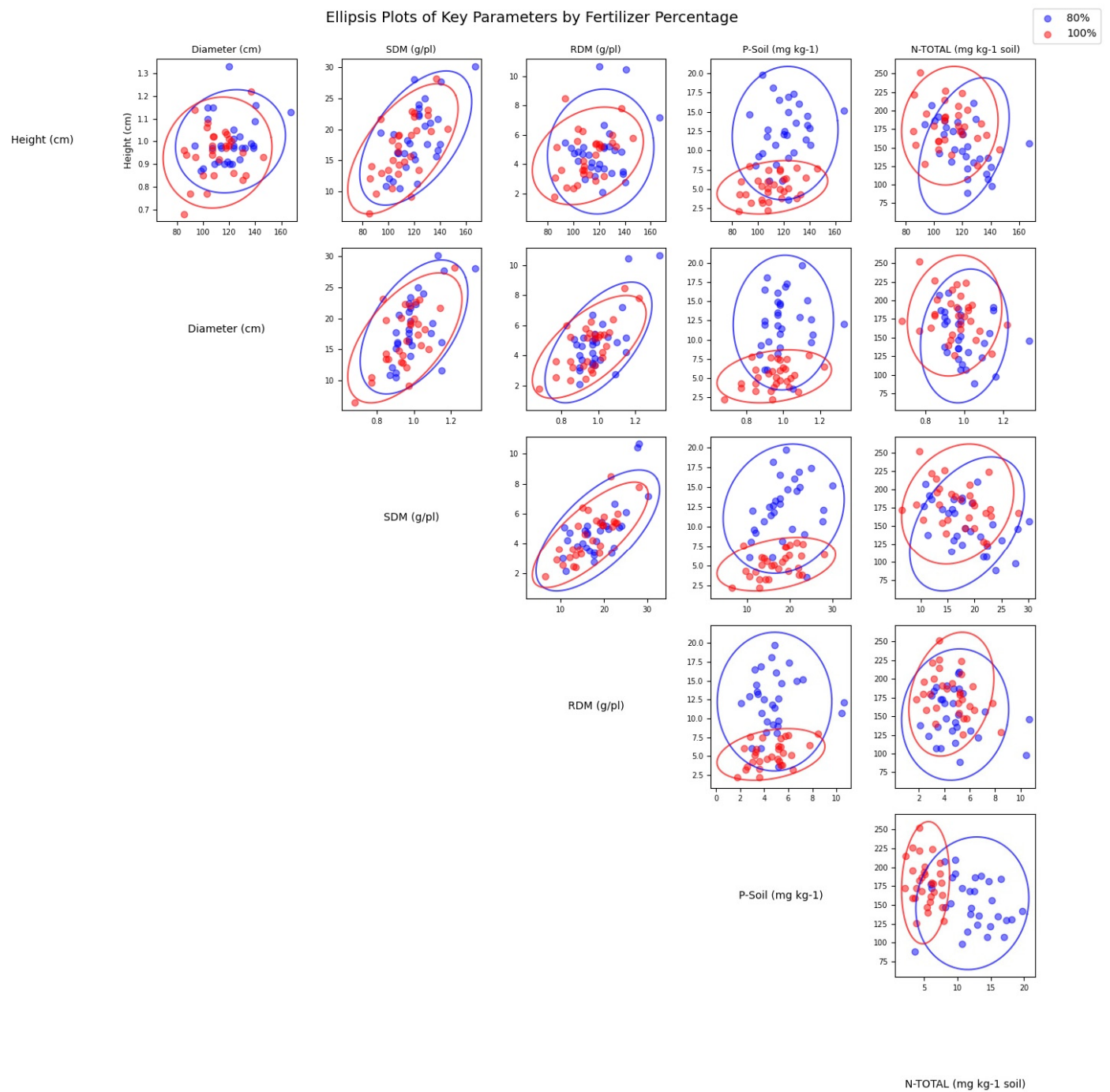


Figure 4. Ellipses plots of key parameters by fertilizer percentage. The blue ellipses indicate fertilizer at 80%, while the red ellipses indicate fertilizer at 100%.

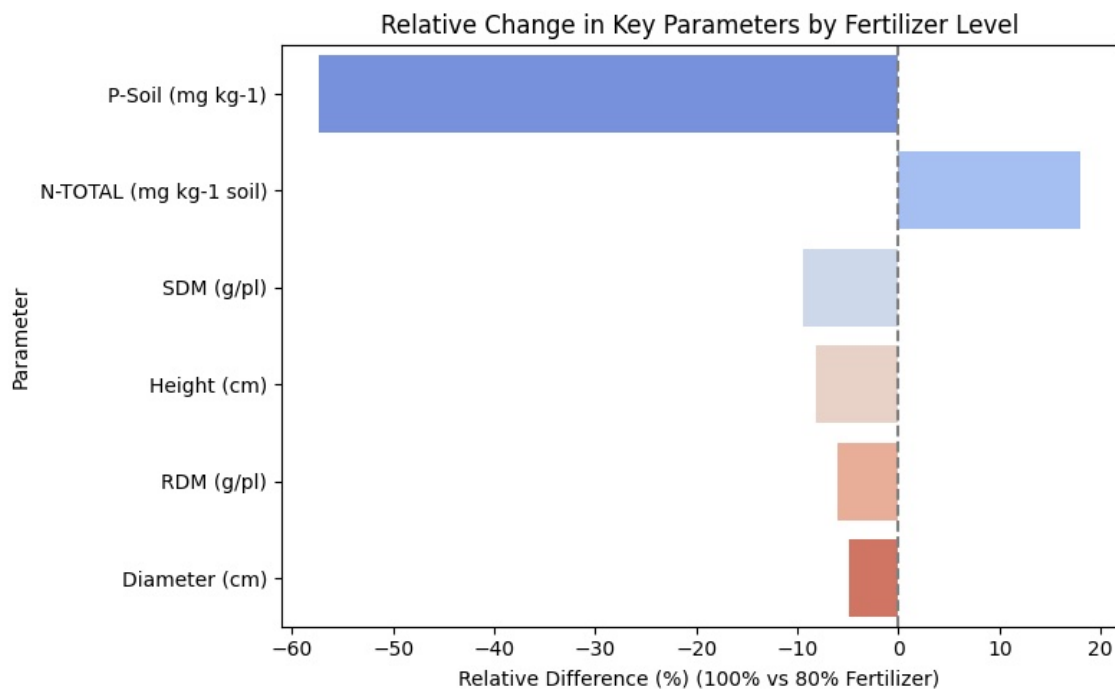


Figure 5. Comparative differences in the assessed parameters between fertilizers applied at 100% and 80%, presented for each parameter.

5. Discussion

In general, there were no statistically significant differences between the treatments with respect to the evaluated parameters, except for height, where T2 exhibited the lowest value. Additionally, treatment T2 demonstrated the highest phosphorus content in shoot dry matter, whereas there was no statistical difference among the other treatments. These results suggest that the application of different treatments had a minimal effect on most of the evaluated parameters. The microorganisms used in this study had several capabilities to promote plant growth. *Bacillus subtilis*, *B. pumilus*, and *B. amyloliquefaciens* can fix atmospheric nitrogen and provide this nutrient to plants, thereby reducing nitrogen fertilizer application. *Bacillus pumilus* demonstrated nitrogen fixation capabilities in nitrogen-free medium, accumulating ammonium and enhancing the growth of *Chlorella vulgaris* [24]. Similarly, *B. pumilus* inoculation improved tomato growth, nitrogen uptake, and soil nitrogenase activity, particularly when combined with nitrogen fertilization [25]. *B. subtilis* and *B. amyloliquefaciens* strains exhibit plant growth-promoting attributes in maize, including increased chlorophyll, carbohydrate, and protein contents [26].

Umapathi [27] demonstrated several positive effects of *Bacillus* spp. on sorghum effects, showing 33% decrease in membrane leakage during drought and a 35% decrease during recovery compared with the control addition, *Bacillus* sp. decreases malondialdehyde (MDA) content. Treated plants showed a 49% reduction in MDA content during drought and a 47% reduction during recovery compared to control plants. Interestingly, the application of *Bacillus* sp. in sorghum promoted the expression of several genes related to plant stress, increasing the tolerance of sorghum to drought stress. Previous studies have demonstrated a significant increase in the length and biomass of sorghum plants, enhancing the efficiency of cadmium (Cd) phytoremediation by augmenting Cd content and accumulation in sorghum, mitigating the stress induced by the reduction in soil mineral nutrients due to Cd + PE composite pollution, increasing the diversity and stability of soil bacterial communities, and promoting phosphorus cycling in the soil by increasing the number of genes related to phosphorus cycling [28].

The positive effect of *Bacillus* sp. inoculation on sorghum crops is associated with the upregulation of gene expression, which enhances stress tolerance. A separate study demonstrated that the inoculation of sorghum seeds with *B. pumilus* yielded several beneficial effects, including seedling protection. Specifically, *B. pumilus* significantly protected sorghum seedlings against *Rhizoctonia solani* infection. In addition, the application of *Bacillus* sp. to sorghum resulted in improved photosynthetic pigment levels. Seeds inoculated with *B. pumilus* exhibited significantly higher contents of photosynthetic pigments, such as chlorophyll a and b, and carotenoids, in the leaves of seedlings compared to the control and uninoculated seedlings. Enhanced root growth was also observed, with bacteria-treated seedlings showing improved root development relative to the control and uninoculated seedlings [29].

In this study, the same treatments were evaluated under two fertilizer conditions: 100% and 80% of the recommended dose. The 20% reduction in fertilization was insufficient for *Bacillus* sp. to exhibit positive effects, likely because of the low stress conditions induced by this reduction. This may account for the lack of significant differences between the treatments receiving these two fertilizer amounts.

Previous studies have demonstrated that the application of *Bacillus* sp. can enhance phosphorus availability in soil because of its capacity to produce enzymes and organic acids that facilitate phosphorus release. However, in the present study, the application of *B. pumilus* to treatment T2 resulted in an increase in N-total and chlorophyll contents [20,30].

Interestingly, the effectiveness of these bacteria in promoting plant growth and nitrogen uptake may depend on the presence of chemical fertilizers. For instance, *Herbaspirillum seropedicae* and *Gluconacetobacter diazotrophicus* increased the nitrogen content in maize leaves and roots under both fertilized and unfertilized conditions, whereas *B. pumilus* and *B. amyloliquefaciens* enhanced phosphorus content [30]. This suggests that these bacteria can be used in conjunction with reduced chemical fertilization doses. Some studies have demonstrated positive effects of *Bacillus* spp. on sorghum crops. *Bacillus subtilis* strains IPACC26 and IPACC30 were found to be particularly effective in promoting plant growth and nitrogen accumulation in sorghum, outperforming other isolates [31]. Similarly, the *Bacillus* sp. PIB1B and PLB1B isolates have demonstrated increased effects on sorghum plant growth in greenhouse tests [32]. These findings highlight the potential of *Bacillus* species as biofertilizer agents for sorghum cultivation.

To our knowledge, this study is the first to evaluate the use of *P. lilacinum* in sorghum (treatment T5). Although the results were not conclusive, further research is necessary to comprehensively assess the impact of this microorganism on sorghum. Technologies such as metagenomic analysis have the potential to enhance the understanding of the beneficial effects of *P. lilacinum* application on sorghum, should such effects occur. Rigobelo et al. [33], utilizing metagenomic analysis, investigated the application of *P. lilacinum* in soybean and determined that the application of this fungus enhanced rhizobium colonization.

Although sorghum has not been specifically mentioned, studies on other crops suggest that *P. lilacinum* could potentially be beneficial for sorghum. This fungus can inhibit plant-pathogenic fungi and nematodes, produce siderophores and indole-3-acetic acid (IAA), and enhance plant development [12]. These properties could be valuable for sorghum cultivation; however, further research is needed to confirm their efficacy in this specific crop.

Although there was no increase in plant growth in the treatments that received 100% of the fertilizer along with microbial inoculants, it is important to note that there was no significant difference between the treatments that received 80% of the fertilizer dose and 100% of the fertilizer dose. This result indicates that these microorganisms can potentially be used to reduce the fertilizer dose.

Despite the effect of microorganism inoculation on sorghum growth, this type of study is important because microbial inoculants, such as plant growth-promoting microorganisms (PGPM), have shown the potential to enhance plant growth, nutrient uptake, and stress tolerance [34]. However, their effectiveness can vary significantly under different biotic and abiotic conditions [35]. Evaluating their performance helps identify which strains are most effective in specific environments and for particular crops, ensuring optimal results in agricultural applications.

Interestingly, some microbial inoculants have shown beneficial secondary effects beyond their primary functions. For instance, plant growth-promoting microorganisms (PGPM) may reduce disease, whereas biological control agents (BCA) can stimulate plant growth even in the absence of pathogens [36]. This multifaceted nature of microbial inoculants highlights the importance of a comprehensive evaluation to fully understand and utilize their potential benefits.

Interesting that, evaluating the effects of microbial inoculants is essential for developing effective and eco-friendly alternatives to chemical fertilizers and pesticides [37]. This allows for the selection of appropriate strains, optimization of application methods, and understanding of their impact on soil microbial communities and overall ecosystem health [38]. This knowledge is crucial for improving agricultural productivity while maintaining ecological stability and reducing the environmental impact.

6. Conclusions

This study assessed the effects of microbial inoculation on plant growth and nutrient uptake under two fertilizer application rates: 80% and 100% of the recommended dosage. Most growth parameters, including plant height and dry matter accumulation, exhibited minimal variation across treatments, with *B. subtilis* yielding the lowest outcomes. Notably, treatments utilizing 80% of the recommended fertilizer rate were comparable to those utilizing 100%, indicating that microbial inoculants may reduce fertilizer requirements without adversely affecting growth. Soil phosphorus and nitrogen levels demonstrated a more pronounced response to variations in fertilizer rates than the physical traits of the plants. Although microbial treatments did not significantly enhance growth, their potential to facilitate reduced fertilizer usage underscores their importance in sustainable agriculture. Future research should focus on optimizing inoculant application methods and timing, examining effects across diverse environmental and stress conditions, assessing long-term impacts on soil health and productivity, and investigating the combined effects of multiple microbial strains. Continued investigation of microbial inoculants is crucial for developing environmentally friendly practices that minimize chemical inputs while maintaining productivity. This study provides a foundation for advancing the use of beneficial microbes to enhance their production efficiency and environmental sustainability.

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