



Bio-inputs as a sustainable alternative for skeletonized coffee plants

Joice Micaela Portugal¹, Jéssica de Fátima Portugal², Victor Vieira Simões³, Ligiane Aparecida Florentino⁴, Tayla Évellin de Oliveira⁵

¹ Universidade Professor José do Rosário Vellano, UNIFENAS. E-mail: joice.micaela@aluno.unifenas.br

² Universidade Professor José do Rosário Vellano, UNIFENAS. E-mail: jessica.portugal@aluno.unifenas.br

³ Universidade Professor José do Rosário Vellano, UNIFENAS. E-mail: victor.simoes@aluno.unifenas.br

⁴ Universidade Professor José do Rosário Vellano, UNIFENAS. E-mail: ligiane.florentino@unifenas.br

⁵ Universidade Professor José do Rosário Vellano, UNIFENAS. E-mail: tayla.oliveira@unifenas.br

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Abstract

Coffee farming is crucial for Brazil, the world leader in coffee production and exportation. Strategies like the “safra zero”, which involve practices such as skeleton pruning, have been adopted to rejuvenate coffee plants and optimize production in alternate years. This contributes to reducing costs and labor demand. Faced with the environmental and economic challenges of coffee cultivation, bio-inputs stand out as a sustainable alternative in crop management, especially during vegetative recovery phases. Therefore, this study was developed to evaluate the effects of applying bio-inputs to coffee plants subjected to skeleton pruning. The experiment was carried out using a randomized block design, in split plots, with eight treatments: five bacterial strains (UNIFENAS 100-94, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus megaterium*, and *B. subtilis*), organized into six combinations, plus a treatment with *A. brasilense* and a control, with four replicates. Applications occurred in March, April, August, and September. Plant height, plagiotropic branch length, and the number of buds were evaluated every 30 days. At the end, soil and leaf chemical analyses were also performed. Mix 2 (UNIFENAS 100-94 + *P. fluorescens*) reached the best performance, with increases in plant height, plagiotropic branch length, and number of buds. All analyzed variables evolved over time. Nutrient levels increased with the use of bio-inputs. It is concluded that these inputs have potential in the post-pruning management of coffee plants.

Keywords: Coffee cultivation. Biological inputs. Beneficial microorganisms. Pruning.

Introduction

Coffee farming is one of the most significant agricultural activities in Brazil, solidifying the country's position as the world's largest coffee producer and exporter. Brazilians are also among the top consumers of coffee, ranking second globally. This strong national and international presence highlights the crop's importance to the economy, especially in states like Minas Gerais, where production is historically well-established. In this context, adopting management practices that not only increase productivity but also improve coffee quality and the sustainability of production systems is essential (DÚRAN *et al.*, 2017; ARAÚJO *et al.*, 2023).

Among the practices used by coffee growers, the pruning system known as “safra zero” has gained prominence due to its efficiency in crop

management. This technique involves drastic pruning of plants in alternate years, which eliminates harvesting costs and reduces labor demand in the non-production year. Additionally, this system promotes plant renewal, controls the biennial cycle of the crop, and restores vegetative vigor, positively impacting productivity in subsequent years (NADALETI *et al.*, 2018; MAIA *et al.*, 2020).

Parallel to physical management practices, there is growing interest in using bio-inputs as a sustainable alternative for coffee cultivation. The use of biological inputs based on microorganisms has shown promise in addressing the environmental and economic challenges faced by modern coffee farming. These inputs contribute to plant development, increase nutrient absorption efficiency, and induce resistance against pests, diseases, and abiotic stresses such as water

scarcity. Furthermore, they represent an important tool in reducing the use of chemical pesticides, aligning production with the growing demand for sustainable practices (VALENTE *et al.*, 2025).

Among the most studied bio-inputs are plant growth-promoting rhizobacteria (PGPR), such as *Azospirillum brasilense*, *Pseudomonas fluorescens*, and species from the *Bacillus* genus. These microorganisms have demonstrated the ability to promote plant growth, improve root development, increase nutrient availability in the soil, and assist in the biological control of pathogens (NAIK *et al.*, 2008; SANTOS *et al.*, 2021a; ETESAMI *et al.*, 2023). Thus, the inoculation of bacterial strains in coffee plantations emerges as a promising strategy to optimize plant development, reduce reliance on chemical inputs, and ensure greater sustainability and economic return for the producer. Given this, the present study was developed to evaluate the effects of applying bio-inputs to coffee plants subjected to skeleton pruning.

Material and methods

The experiment was carried out at the Padre Rocha site in Campos Gerais, in the state of Minas Gerais, Brazil (21°23'32" S, 45°71'52" W and 843 m altitude), in an area planted with Mundo Novo coffee in 2011, with 3 m spacing between rows and 1.20 m between plants, totaling 1.2 hectares and an average annual yield of 45 bags per hectare. With the crop

completing seven years in 2018, management known as "zero harvest" began between August and September. The region has a tropical climate, classified as Cwb according to the Köppen classification, with average temperatures and precipitation during the study of 21.0 °C and 1,455 mm, respectively. The soil in the area is classified as an Oxisol with a clay texture, and at the beginning of the study, it had the following chemical characteristics: pH (CaCl₂) = 5.6; organic matter = 4.28 dag kg⁻¹ g; P (resin) = 50.4 mg dm⁻³; K = 4.91 mmol_c dm⁻³; Ca²⁺ = 4.7 cmol_c dm⁻³; Mg²⁺ = 1.8 cmol_c dm⁻³; Al³⁺ = 0 cmol_c dm⁻³; H + Al = 1.9 cmol_c dm⁻³; SB = 7 cmol_c dm⁻³; t = 7 cmol_c dm⁻³; T = 8.9 cmol_c dm⁻³; V = 78.7 %; m = 0 %; P-rem = 18.3 mg L⁻¹.

The treatments consisted of the combined application of five microorganisms, one of which was a bacterial isolate obtained and characterized at the Soil Microbiology Laboratory (LMS) of the Professor Edson Antônio Velano University (UNIFENAS), identified as UNIFENAS 100-94, and four microorganisms from commercial products: *A. brasilense* Ab-V5, *P. fluorescens* CCTB 03, *Bacillus megaterium* CNPMS B119, and *B. subtilis* CCTB 04, organized into six different combinations. The choice to use these microorganisms together took into account the different functions performed by each one. A treatment with only *A. brasilense* and a control treatment, in which no inoculation was performed, were also included (Table 1).

Table 1. Combinations of bacterial strains used to compose the mixes.

Identification	Applied strains
Mix 1	UNIFENAS 100-94 + <i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i>
Mix 2	UNIFENAS 100-94 + <i>P. fluorescens</i>
Mix 3	UNIFENAS 100-94 + <i>A. brasilense</i> + <i>Bacillus subtilis</i>
Mix 4	UNIFENAS 100-94 + <i>B. subtilis</i>
Mix 5	UNIFENAS 100-94 + <i>A. brasilense</i> + <i>Bacillus megaterium</i>
Mix 6	UNIFENAS 100-94 + <i>B. megaterium</i>

Source: authors (2024).

The experimental design used was a Randomized Block Design (RBD) with split plots over time. It included eight treatments and four repetitions, totaling 32 experimental plots. Each plot consisted of six plants, corresponding to an area of 21.6 m², resulting in a total area of 691.2 m² in the experiment. The four central plants were considered for data collection, while the two side plants were used as borders to avoid interference between treatments.

Inoculant preparation was carried out at the Soil Microbiology Laboratory (LMS) of the Professor Edson Antônio Velano University (UNIFENAS). Bacterial strains were cultivated on solid culture media for 48 hours at 28°C: Medium 79 (FRED, WAKSMAN, 1928) for UNIFENAS 100-94, *A. brasilense*, and *P. fluorescens*, and nutrient agar (0.5 % peptone, 0.3 % meat extract, 0.5 % NaCl, and 1.5 % agar) for *Bacillus* genus strains. The colonies were then transferred to their respective liquid media (79 and nutrient broth) and cultured for the same period and temperature until they reached the log phase, with an approximate concentration of 10⁸ cells mL⁻¹ estimated by counting in culture medium and expressed in colony-forming units per milliliter (CFU mL⁻¹).

The applications were carried out via soil, using a backpack sprayer, with 320 mL of inoculant applied for each microorganism present in the treatment. In combination treatments, the doses were added together, but always diluted in a volume of 2.5 liters of spray per treatment, so that the total volume of spray remained constant regardless of the number of microorganisms applied. In total, four applications were carried out, distributed across the months of March, April, August, and September.

An initial assessment was carried out at time zero, before the application of treatments, and used as a control. The other assessments were carried out every 30 days after the applications, evaluating the height of the plants and the length of the plagiotropic branches located in the upper

middle third, i.e., those with productive potential for the following harvest, previously marked with nylon ties. The height and length of the branches were measured with a graduated tape measure, and the number of buds present on these same branches was also counted.

At the end of the experiment, chemical analyses were performed on the soil (phosphorus and potassium) and leaves (macro and micronutrients). Soil samples were collected from a depth of 0 to 20 cm, with two subsamples taken per plot to form a composite sample; in total, three composite samples were obtained for analysis. The leaves were collected from the middle third of the plants, between the third and fourth pair of leaves. All samples were then sent to the Chemical Analysis Laboratory of the Guaxupé Regional Coffee Growers Cooperative (COOXUPÉ).

The data were subjected to analysis of variance (ANOVA). For the qualitative factor, the means were compared using the Scott-Knott test, at 5 % significance. For the quantitative factor, regression models were adjusted. Statistical analyses were performed using SISVAR software (FERREIRA, 2018).

Results and discussion

There was no interaction between inoculant and application days for plant height, plagiotropic branch length, and number of buds. However, both factors had significant effects when evaluated separately. For plant height, increases were observed with the inoculation of *A. brasilense*, Mix 1, Mix 2, Mix 4, and Mix 6, indicating a positive effect of the isolated application of *A. brasilense* and the combinations used in the mixes (Table 2). This result may be related to the production of phytohormones, especially indoleacetic acid (IAA), in addition to the greater absorption of nutrients promoted by microorganisms (DIAZ *et al.*, 2019; SANTOS *et al.*, 2021a).

Table 2. Means of plant height, plagiotropic branch length, and number of buds in skeletonized coffee plants after bio-input application.

Inoculation	Plant height (m)	Plagiotropic branch length (cm)	Number of buds (units)
<i>A. brasilense</i>	3.03 A	58.86 B	13.10 B
Mix 1	3.04 A	57.56 B	13.18 B
Mix 2	3.01 A	66.47 A	14.85 A
Mix 3	2.91 B	61.55 B	13.33 B
Mix 4	3.07 A	62.73 A	13.07 B
Mix 5	2.84 B	59.58 B	13.60 B
Mix 6	3.11 A	63.92 A	13.11 B
Controle	2.88 B	61.05 B	13.75 B
CV %	6.50	8.70	8.68

Means followed by the same letters do not differ significantly from each other by the Scott-Knott test ($p < 0.05$). Mix 1: UNIFENAS 100-94 + *Azospirillum brasilense* + *Pseudomonas fluorescens*; Mix 2: UNIFENAS 100-94 + *P. fluorescens*; Mix 3: UNIFENAS 100-94 + *A. brasilense* + *Bacillus subtilis*; Mix 4: UNIFENAS 100-94 + *B. subtilis*; Mix 5: UNIFENAS 100-94 + *A. brasilense* + *Bacillus megaterium*; Mix 6: UNIFENAS 100-94 + *B. megaterium*; CV (%): coefficient of variation.

Source: authors (2024).

Ferreira *et al.* (2022), when inoculating *A. brasilense*, *P. fluorescens*, and a bacterium of the genus *Bacillus* in coffee seedlings, observed an increase in plant height 120 days after application, with emphasis on inoculation with *A. brasilense*, which provided the best results. Similarly, Lima *et al.* (2023) also observed greater height in coffee seedlings inoculated with *A. brasilense* after 150 days, reinforcing the potential of this bacterium for the initial development of the crop. On the other hand, Silva *et al.* (2020), when evaluating the height of coffee seedlings 150 days after inoculation with *A. brasilense*, did not observe significant differences, which shows that the effects of inoculation may vary according to experimental conditions.

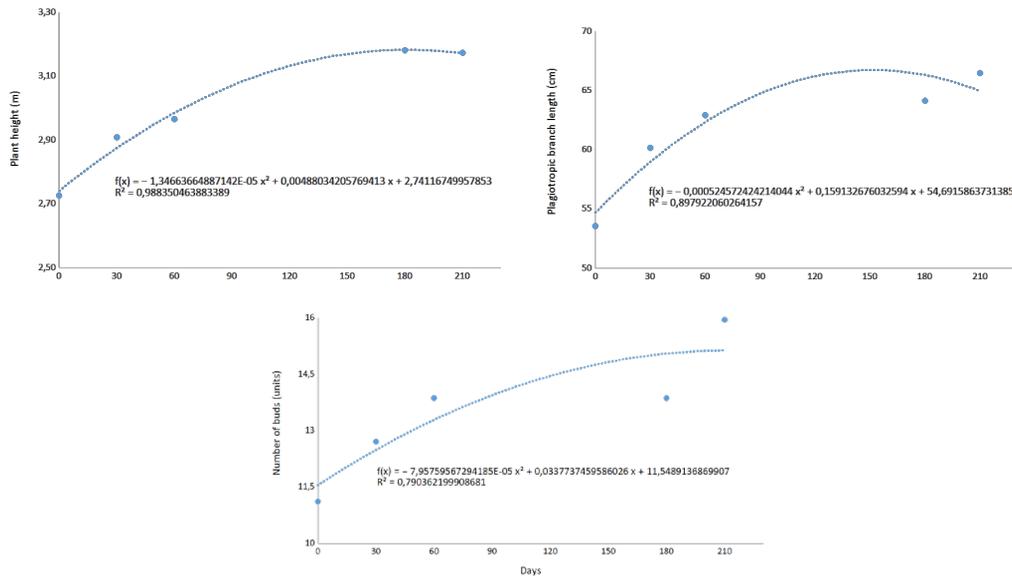
It is known that PGPR benefit plants through direct and indirect mechanisms, but the effectiveness of inoculation is directly related to the interaction between the microorganism, the plant, and the environment. In addition, factors such as the method and timing of application, as well as the compatibility between the strains used, also influence the results obtained with the

use of these biotechnologies (URGILES-GÓMEZ *et al.*, 2021).

As for the length of the plagiotropic branches, an increase was observed in the treatments Mix 2, Mix 4, and Mix 6. The number of buds was also higher in the treatment Mix 2 (Table 2). Plagiotropic branches and buds are an important indicator of the productive capacity of coffee trees, as it is in these structures that flowering and fruiting occur (OLIVEIRA NETO *et al.*, 2022). To ensure the proper development of these structures, it is essential to manage the crop efficiently, including pruning, fertilization, and pest and disease control. In this context, the fact that Mix 2, composed of the strains UNIFENAS 100-94 and *P. fluorescens*, promoted an increase in the number of buds in skeletonized plants is of great practical relevance, since studies indicate the potential of these strains to stimulate plant growth and solubilize nutrients (VYAS; GULATI, 2009; FLORENTINO *et al.*, 2017; SILVA *et al.*, 2019).

There was a progressive increase in plant height, plagiotropic branch length, and number of buds over the course of the days (Figure 1).

Figure 1. Plant height, length of plagiotropic branches, and number of buds over the days following application.



Source: authors (2024).

The progressive increase in these parameters is an expected behavior, as they develop naturally during the vegetative recovery phase after skeletonization. The progress of development over the days reflects the resumption of active growth of productive branches, with greater availability of photoassimilates and continuous tissue elongation. Studies report that this growth pattern is associated with the physiological dynamics of the crop, which tends to intensify the emission and expansion of branches and buds after pruning interventions or controlled stress (MATIELO *et al.*, 2007; COSTA *et al.*, 2022).

The average levels of phosphorus (P) and potassium (K) in the soil are presented in Table 3. The highest P content was observed in the Mix 3 treatment, while the highest K content was associated with the application of the *A. brasilense* strain.

These results reflect the ability of the inoculated microorganisms to solubilize nutrients in the soil. P and K are essential macronutrients for coffee plant development and productivity, and using solubilizing microorganisms has been identified as a promising strategy to increase the

efficiency of these elements' availability, reducing reliance on synthetic fertilizers (NAVARRO *et al.*, 2021; ARAÚJO *et al.*, 2024). Literature highlights the potential of *P. fluorescens* (VYAS; GULATI, 2009), *Azospirillum* spp. (CASSÁN *et al.*, 2020), *B. subtilis* (KUMARI *et al.*, 2024), and *B. megaterium* (VELLOSO *et al.*, 2020) in P solubilization, and of the UNIFENAS 100-94 strain in K solubilization (FLORENTINO *et al.*, 2017). However, it's worth noting that these studies were conducted with isolated microorganisms. In the current experiment, the microorganisms were used in combination, with the exception of *A. brasilense*, which was applied individually. Its response indicated greater efficiency in K availability, differing from the behavior described in the literature regarding P.

The highest foliar nitrogen (N) and phosphorus (P) contents were observed with the application of Mix 5. The highest K contents were found in Mix 1, Mix 3, and Mix 6 treatments, and the highest calcium (Ca), magnesium (Mg), and sulfur (S) contents were in Mix 4 (Table 4).

The higher foliar N and P contents observed in the Mix 5 treatment might be related to

Table 3. Mean phosphorus (P) and potassium (K) contents in soil at 210 days after bio-input application in skeletonized coffee plants.

Inoculation	P (resin)	K (NH ₄ Cl)
	mg/dm ³	mmol/dm ³
<i>A. brasilense</i>	55.00 G	7.96 A
Mix 1	74.33 D	6.83 C
Mix 2	76.66 C	6.16 D
Mix 3	100.00 A	6.83 C
Mix 4	72.66 D	7.06 B
Mix 5	59.66 F	5.23 E
Mix 6	81.66 B	7.30 B
Control	70.33 E	6.63 C
CV (%)	2.14	2.69

Means followed by the same letters do not differ significantly from each other by the Scott-Knott test ($p < 0.05$). Mix 1: UNIFENAS 100-94 + *Azospirillum brasilense* + *Pseudomonas fluorescens*; Mix 2: UNIFENAS 100-94 + *P. fluorescens*; Mix 3: UNIFENAS 100-94 + *A. brasilense* + *Bacillus subtilis*; Mix 4: UNIFENAS 100-94 + *B. subtilis*; Mix 5: UNIFENAS 100-94 + *A. brasilense* + *Bacillus megaterium*; Mix 6: UNIFENAS 100-94 + *B. megaterium*; CV (%): coefficient of variation.

Source: authors (2024).

the combined action of *A. brasilense* and *B. megaterium* strains. The *A. brasilense* strain contributes to biological N fixation (FUKAMI *et al.*, 2018) and also to P solubilization

(CASSÁN *et al.*, 2020), while *B. megaterium* primarily acts in P solubilization (VELLOSO *et al.*, 2020), favoring its availability to plants. Generally, the treatments provided adequate

Table 4. Mean contents of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) in leaves of skeletonized coffee plants, evaluated at 210 days after bio-input application.

Inoculation	N	P	K	Ca	Mg	S
	g/kg					
<i>A. brasilense</i>	27.94 D	1.13 F	25.14 B	9.57 G	2.71 E	2.18 D
Mix 1	29.16 C	1.27 E	26.07 A	10.36 D	2.83 D	2.37 C
Mix 2	29.05 C	1.43 D	23.25 C	10.91 C	2.83 D	2.43 B
Mix 3	31.19 B	1.55 B	26.07 A	10.12 E	2.85 D	2.35 C
Mix 4	29.89 C	1.51 C	24.91 B	11.96 A	3.16 A	2.54 A
Mix 5	33.30 A	1.84 A	24.60 B	9.33 H	2.75 E	2.46 B
Mix 6	29.24 C	1.24 E	25.97 A	11.60 B	2.96 C	2.35 C
Control	29.58 C	1.44 D	25.11 B	9.92 F	3.07 B	2.39 C
CV (%)	1.31	1.34	1.27	1.06	1.17	1.02

Means followed by the same letters do not differ significantly from each other by the Scott-Knott test ($p < 0.05$). Mix 1: UNIFENAS 100-94 + *Azospirillum brasilense* + *Pseudomonas fluorescens*; Mix 2: UNIFENAS 100-94 + *P. fluorescens*; Mix 3: UNIFENAS 100-94 + *A. brasilense* + *Bacillus subtilis*; Mix 4: UNIFENAS 100-94 + *B. subtilis*; Mix 5: UNIFENAS 100-94 + *A. brasilense* + *Bacillus megaterium*; Mix 6: UNIFENAS 100-94 + *B. megaterium*; CV (%): coefficient of variation.

Source: authors (2024).

levels of these nutrients for coffee cultivation in Southern Minas Gerais (RIBEIRO *et al.*, 1999), with the exception of the isolated *A. brasilense* application, which resulted in lower foliar contents. This outcome reinforces the importance of associating microorganisms with different functions. Furthermore, the low P contents in leaves when *A. brasilense* was applied individually corresponded with lower nutrient availability in the soil, indicating that soils with lower nutrient content effectively provide less of these elements to plants.

Although the highest foliar K contents were observed in Mix 1, Mix 3, and Mix 6 treatments, all treatments reached values within the threshold considered adequate for coffee plants in the region, as established by Ribeiro *et al.* (1999). Best results obtained in the mentioned mixtures may be attributed to the presence of the UNIFENAS 100-94 strain, recognized for its K solubilizing capacity (FLORENTINO *et al.*, 2017).

While most studies on PGPRs focus on the availability of N, P, and K, there is literature

evidence associating these strains with increased absorption of Ca, Mg, and S. Galindo *et al.* (2015) observed higher foliar Ca, Mg, and S contents in wheat plants inoculated with *A. brasilense*. Similarly, Pacheco *et al.* (2024) also found increases in the foliar contents of these nutrients in maize after inoculation with the same bacterium. On the other hand, Santos *et al.* (2021b), evaluating the inoculation of *B. subtilis* and *B. megaterium* in white oats, only observed an increase in S contents. Picazevicz *et al.* (2020) suggest that this increase might be associated with the stimulation of root growth promoted by PGPRs, favoring the absorption of nutrients present in the soil.

Regarding micronutrients, the highest foliar boron (B) and iron (Fe) contents were observed with the application of Mix 2. The highest copper (Cu) and zinc (Zn) contents were found in Mix 5, and manganese (Mn) in Mix 6 (Table 5).

Overall, the foliar Cu, Mn, and Zn contents remained within the adequate threshold for coffee plants cultivated in Southern Minas Gerais. Notably, Mn levels in Mix 4 and Mix 6 treatments were

Table 5. Mean contents of micronutrients zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and boron (B) in leaves of skeletonized coffee plants, evaluated at 210 days after bio-input application.

Inoculation	B	Cu	Fe	Mn	Zn
	----- mg/kg -----				
<i>A. brasilense</i>	32.36 F	19.03 E	326.51 E	163.09 D	15.19 E
Mix 1	41.30 B	20.28 C	331.94 C	160.20 E	18.74 B
Mix 2	42.80 A	18.21 F	359.41 A	163.88 C	13.65 G
Mix 3	34.56 D	17.52 G	299.21 H	159.06 F	13.35 H
Mix 4	40.95 C	15.59 H	327.73 D	186.69 B	14.44 F
Mix 5	33.63 E	26.38 A	302.48 G	148.14 G	19.42 A
Mix 6	34.81 D	20.70 B	316.52 F	189.20 A	17.76 C
Control	34.74 D	19.35 D	339.73 B	141.57 H	15.73 D
CV%	0.49	0.90	0.16	0.23	0.41

Means followed by the same letters do not differ significantly from each other by the Scott-Knott test ($p < 0.05$). Mix 1: UNIFENAS 100-94 + *Azospirillum brasilense* + *Pseudomonas fluorescens*; Mix 2: UNIFENAS 100-94 + *P. fluorescens*; Mix 3: UNIFENAS 100-94 + *A. brasilense* + *Bacillus subtilis*; Mix 4: UNIFENAS 100-94 + *B. subtilis*; Mix 5: UNIFENAS 100-94 + *A. brasilense* + *Bacillus megaterium*; Mix 6: UNIFENAS 100-94 + *B. megaterium*; CV (%): coefficient of variation.

Source: authors (2024).

above the sufficiency range. For Fe, all treatments showed levels above the considered adequate threshold. In contrast, B levels were mostly below the recommended range, except for Mix 1 and Mix 2 treatments, which presented values within the adequate range (RIBEIRO *et al.*, 1999).

The positive effects of PGPRs on micronutrient absorption are linked to various mechanisms. These include the production of siderophores, low molecular weight chelating molecules primarily produced under low soil Fe availability, which promote its solubilization and facilitate plant uptake (GHAVAMI *et al.*, 2017; FERREIRA *et al.*, 2019). Additionally, these compounds can also form stable complexes with Cu and Zn (NEUBAUER *et al.*, 2000), expanding the availability of these nutrients. Bacteria from the *Bacillus* and *Pseudomonas* genera complement this process through the production of organic acids, capable of solubilizing micronutrients (MUMTAZ *et al.*, 2019; MISHRA *et al.*, 2025). Coupled with these mechanisms is the previously mentioned stimulation of root growth, which increases the soil exploration area and favors micronutrient absorption by roots, resulting in higher foliar contents.

Conclusions

The application of bio-inputs to coffee plants subjected to skeleton pruning had a positive effect on the vegetative development and nutritional status of the crop. Mix 2 (UNIFENAS 100-94 + *P. fluorescens*) performed best, with increases in plant height, plagiotropic branch length, and number of buds. Nutrient levels in the soil and leaves were also higher in the treatments with bio-inputs, indicating improved plant nutrition.

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