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Co-Inoculation of Phosphate-Solubilizing Bacteria and Rhizobia Increases Phosphorus Availability and Promotes the Development of Forage Legumes

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Abstract: Tropical grassland soils, especially those with alkaline properties, often exhibit limited phosphorus availability due to its precipitation in insoluble forms. Phosphate-solubilizing bacteria (PSB) and rhizobia have demonstrated their potential to enhance the availability of this nutrient and promote the growth of forage legumes. This study, conducted under controlled conditions in a mesh house, evaluated the effect of co-inoculation with PSB, including *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *Enterobacter* sp. Sfcm-014-02 and Sfcm-054-06, along with rhizobia (*Ensifer teranga* R1-012-02 and *Bradyrhizobium glycinis* Rcm-025-01), under different levels of phosphorus fertilization on the legumes *Leucaena leucocephala* and *Centrosema macrocarpum*. The results indicate significant increases in various growth parameters, such as chlorophyll levels (SPAD), biomass (dry weight of roots and aerial parts) (mg), the foliar phosphorus concentration (ppm), and the concentration of available phosphorus in the soil, particularly under low-phosphorus fertilization conditions. The highest level of available phosphorus in the soil was achieved with 75% of the recommended fertilization dose, resulting in concentrations of 13.73 ppm for *L. leucocephala* and 7.69 ppm for *C. macrocarpum*, representing increases in phosphorus availability of 170.81% and 240.27%, respectively, compared with no fertilization or inoculation. These findings suggest that the co-inoculation of PSB and native rhizobia is a promising strategy to enhance the biomass productivity and mineral content of forage in tropical grazing systems, especially under phosphorus-limited conditions.

Keywords: foliar phosphorus; chlorophyll level; aerial dry biomass; phosphate solubilization; tropical grazing

1. Introduction

Phosphorus is an essential macronutrient for plant growth and development, playing a crucial role in processes such as DNA, RNA, and ATP synthesis, photosynthesis, respiration, energy transfer, signal transduction, macromolecule biosynthesis, and biological nitrogen fixation (BNF) in legumes [1]. However, in many soils, phosphorus availability

is limited due to its fixation in insoluble forms, which restricts agricultural productivity [2,3]. Although synthetic phosphate fertilizers can mitigate this issue, their use presents economic and environmental challenges, including soil degradation, runoff pollution, and eutrophication [4,5]. Additionally, high-quality natural phosphate reserves are finite and are expected to be depleted in the coming decades, posing a significant challenge to long-term agricultural sustainability [6,7].

Soil microorganisms play a vital role in plant nutrition by facilitating nutrient absorption through processes such as mineralization and the solubilization of compounds inaccessible to plants [8]. Among these microorganisms, phosphate-solubilizing bacteria (PSB) are notable for their ability to solubilize insoluble phosphorus forms such as tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) via mechanisms including organic acid production, proton release, enzyme secretion, and siderophore synthesis [9–11]. Various bacterial genera, including *Micrococcus* sp., Gram-positive cocci [12], *Agrobacterium* sp., and Gram-negative bacilli [13], stand out as plant growth promoters [14,15]. The co-inoculation of PSB with rhizobia, which fix atmospheric nitrogen in symbiosis with legumes, can produce synergistic benefits that optimize phosphorus and nitrogen availability in the soil, promoting plant growth and resilience while reducing dependence on chemical fertilizers [16].

Using biofertilizers containing PSB is an ecological and sustainable alternative to synthetic fertilizers, promoting soil fertility recovery [17,18]. Legumes are more affected by phosphorus deficiencies due to the high energy demand for BNF during symbiosis with rhizobia, especially for reducing molecular nitrogen to ammonia (NH_3) [19]. Phosphorus deficiencies can compromise nodulation and BNF, affecting vital processes such as photosynthesis, respiration, and vegetative growth [20,21].

In temperate and tropical regions, where livestock production is important, perennial forage legumes are essential for agricultural sustainability [22]. The co-inoculation of PSB and rhizobia has proven to be an effective strategy to enhance phosphorus availability in low-fertility soils [16,23]. For example, the genus *Centrosema* from the subfamily Papilionoideae adapts well to various arid tropical environments, including acidic soils and areas affected by drought or seasonal flooding, where phosphorus solubilization is critical [24]. Similarly, the genus *Leucaena*, known for its high biomass production, benefits from co-inoculation with PSB, improving its growth in nutrient-poor soils and facilitating BNF in root nodules, which is essential for its development in degraded soils [25]. *Leucaena leucocephala*, one of the most widely distributed species of this genus, is valued in agroforestry systems for its ability to improve soil fertility and its high value as livestock forage [26,27]. This approach represents an ecological and sustainable alternative to chemical fertilizers, which can improve both soil health and agricultural productivity. Research highlights the importance of applying a co-inoculation of PSB and rhizobia as a key strategy for rehabilitating degraded soils, especially in arid tropical areas vulnerable to climate change, where forage legumes are essential for agricultural and livestock sustainability.

This research aimed to evaluate the effect of the co-inoculation of PSB and rhizobia in symbiosis with two forage legume species, *Leucaena leucocephala* and *Centrosema macrocarpum*, focusing on nutritional status (chlorophyll level and foliar P concentration), growth performance (plant height, dry biomass of aerial parts and roots, and root length), and phosphorus dynamics in the soil (P concentration) in a controlled environment.

2. Materials and Methods

2.1. Biological Material

Bacterial strains from the strain bank of the Agricultural Microbiology Laboratory 'Raúl Ríos Reátegui', affiliated with the Faculty of Agricultural Sciences at the National University of San Martín (UNSM), were used. The bacterial strains employed included *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, *Enterobacter* sp. Sfcm-014-02, and *Enterobacter* sp. Sfcm-054-06, which had previously been characterized as tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) solubilizers. Additionally, rhizobial strains were used, specifically *Ensifer teranga* R1-012-02 and *Bradyrhizobium glycinis* Rcm-025-01 (see Table 1).

Table 1. Phosphate-solubilizing bacterial strains and rhizobia isolated from the rhizosphere of cover legumes in the Carañayacu region, Cuñumbuque district, San Martín, Peru.

Strains	Host Legume	Identified Strain/ Access Number in NCBI GenBank
Phosphate-solubilizing bacterial strains		
Sfcm-014-01	<i>Centrosema macrocarpum</i>	<i>Micrococcus</i> sp. Sfcm-014-01/PQ215700
Sfl-043-09	<i>Leucaena leucocephala</i>	<i>Agrobacterium</i> sp. Sfl-043-09/PP319609
Sfcm-014-02	<i>Centrosema macrocarpum</i>	<i>Enterobacter</i> sp. Sfcm-014-02/PP319605
Sfcm-054-06	<i>Centrosema macrocarpum</i>	<i>Enterobacter</i> sp. Sfcm-054-06/PP319604
Rhizobial strains		
RI-012-02	<i>Leucaena leucocephala</i>	<i>Ensifer teranga</i> RI-012-02/PQ345348
Rcm-025-01	<i>Centrosema macrocarpum</i>	<i>Bradyrhizobium glycinis</i> Rcm-025-01/PQ356800

The strains were reactivated using the streaking and purification technique, following the protocol described by Liu et al. [28]. The PSB strains were streaked on Petri dishes containing tryptone soy agar (TSA) medium, consisting of pancreatic digest of casein (15 g), papaic digest of soybean (5 g), sodium chloride (5 g), Agar-Agar (15 g), and distilled water (1000 mL), adjusted to a pH of 6.9. Meanwhile, the rhizobial strains were inoculated on plates with yeast extract mannitol (YEM) medium, composed of mannitol (5.0 g), K₂HPO₄ (0.5 g), MgSO₄·7H₂O (0.2 g), NaCl (0.1 g), yeast extract (0.5 g), Agar-Agar (15.0 g), and distilled water (1000 mL), adjusted to a pH of 7.0. The PSB plates were incubated at 30 °C for 48 h, while the rhizobial plates were incubated between 120 and 144 h at the same temperature. The purity of the cultures was then assessed before performing new subcultures for the experiments.

2.2. Antagonism Test

To begin, a pre-inoculum was prepared by inoculating the refrigerated strains into tubes containing 3 mL of tryptone soy broth (TSB) for the PSB strains and into YEM broth for the rhizobial strains. The cultures were incubated at 30 °C for 48 to 144 h, depending on the growth time of each strain, under constant agitation at 150 rpm. YEM agar plates were prepared three days before the inhibition assay, ensuring they were dry and free from contaminants. The plates were then inoculated with 100 µL of YEM broth containing rhizobia in their active growth phase, with uniform streaking. The inoculated plates were left to dry for 15 min. Next, the plates were divided into four quadrants, where 5 µL of TSB broth containing the PSB strains were inoculated in each quadrant. The plates were incubated in an inverted position at 28 °C for 48 h for the PSB strains and 72 h for the rhizobial strains. A positive antagonism result was indicated by the presence of an inhibition zone around the PSB colonies, suggesting a possible inhibitory interaction between the strains. A negative result was characterized by the absence of inhibited growth in these zones, suggesting compatibility between the strains.

2.3. Quantification of Bacterial Growth

The inoculum was prepared from strains stored under refrigeration. A loopful of each strain was taken and inoculated into tubes containing 3 mL of TSB for PSB and YEM broth for the rhizobia. The cultures were incubated at 30 °C under agitation at 150 rpm for 48 h for PSB and 72 h for rhizobia. Serial dilutions of the cultures were then made, achieving an approximate concentration of 1×10^9 cells mL⁻¹. Eight microtubes were prepared for the dilutions, each with 900 µL of a 0.85% sterile saline solution. Subsequently, 100 µL of the initial culture was diluted in ten steps, using sterile micropipettes for each transfer. Five 250 mL Erlenmeyer flasks were used for each strain to calculate the bacterial generation time, each containing 100 mL of TSB or YEM broth. Starting from a 10⁻² dilution of the culture, equivalent to 1×10^6 cells mL⁻¹, 20 µL was inoculated into each flask. Viable counts were performed at intervals of 4 to 6 h for 72 h for PSB and rhizobia of the genus

Enterobacter, and every 12 h for 120 h for *Bradyrhizobium*, beginning from time 0. For the count, 30 μL from each dilution was plated on agar plates previously divided into eight equal sections and labeled with the corresponding dilutions, duplicating the samples. The plates were left to dry for 15 min before incubation at 28 °C for 3 to 5 days [29].

2.4. Experimental Setup and Implementation

The experiment was conducted under semi-controlled conditions in a mesh house at the Faculty of Agricultural Sciences of UNSM, lasting 90 days. The soil was collected from the Carañayacu area, Cuñumbuque district, San Martin, Peru, via random zigzag sampling at 10 equidistant points, approximately 20 m apart. From each point, 1 kg of soil was extracted at a depth of 20 cm [30]. The collected samples were mixed and homogenized to form a composite sample of 1 kg, which was used for physicochemical analysis. The remaining soil was air-dried, crushed, and sieved. Subsequently, 2 kg of soil was packed in polypropylene bags and sterilized by autoclaving (Astell AMA440070, London, UK) at 121 °C for 1 h, in three cycles.

The *L. leucocephala* and *C. macrocarpum* seeds were disinfected by immersion in 70% alcohol, followed by 2.5% sodium hypochlorite. They were then rinsed with sterile water and pre-germinated on filter paper at 28 °C for 2 to 3 days. The PSB and rhizobia inoculants were prepared in flasks with TSB and YEM broth, respectively, and incubated at 30 °C for 24 to 96 h. The optical density ($\text{OD}_{600\text{nm}}$) was adjusted to 1.0, equivalent to approximately 1×10^9 CFU mL^{-1} . Pots were sterilized with 0.1% sodium hypochlorite for 12 h and rinsed with distilled water. The pre-germinated seeds were sown in the pots with 2 kg of sterile soil and inoculated with 0.5 mL of PSB and 0.5 mL of effective rhizobium, both with an $\text{OD}_{600\text{nm}}$ of 1.0. Thinning was performed after germination to leave two plants per pot.

Fertilization included the application of urea, triple superphosphate, and potassium chloride, with specific nitrogen, phosphorus, and potassium (N-P-K) doses of 120-75-60 for *L. leucocephala* and 120-90-100 for *C. macrocarpum*. Phosphorus was applied 10 days before co-inoculation, while nitrogen and potassium were incorporated 10 days after seedling emergence. A second fertilization was performed 45 days after emergence, applying phosphorus at four levels: 0%, 25%, 50%, and 75% of the optimal dose for each legume. Nitrogen was applied only to the positive control treatment, while potassium was evenly distributed across all treatments.

At 90 days post-planting, the following parameters were evaluated: the chlorophyll level (measured with a SPAD 502 Plus meter), plant height (in cm, from the base of the stem to the apex), dry biomass of shoots and roots (in mg, dried at 70 °C for 48 h until reaching a constant weight), root length (in cm, from the base of the stem to the tip of the main root), foliar phosphorus concentration (in ppm, via nitric-perchloric acid digestion and UV spectrophotometer (ThermoFisher, Spectronic 200, Suwa, Japan) readings at 600 nm), and soil phosphorus concentration (in ppm, using Olsen extraction and UV spectrophotometer (ThermoFisher, Spectronic 200, Suwa, Japan) readings at 600 nm).

2.5. Statistical Design

The experiment conducted for each legume species under study (*L. leucocephala* and *C. macrocarpum*) was designed using a completely randomized factorial design. This design included 3 types of bacterial strains (2 PSB and 1 rhizobium) and 4 levels of phosphorus in the form of tricalcium phosphate (0%, 25%, 50%, and 75%). Additionally, experimental controls were incorporated, consisting of a control with rhizobium (*E. terangae* for *L. leucocephala* and *B. glycinis* for *C. macrocarpum*) along with controls for the four tricalcium phosphate levels (0%, 25%, 50%, and 75%). A control with complete fertilization at 100% NPK (nitrogen, phosphorus, and potassium) was also added. In total, the experimental design included 21 treatments, each with 3 replicates, resulting in a total of 63 experimental units per legume species. The results were statistically analyzed using an analysis of variance (ANOVA), followed by a significance test using Fisher's LSD test ($p < 0.05$).

3. Results

3.1. Soil Analysis

The physicochemical analysis of the soil samples was carried out at the Soil and Water Analysis Laboratory of the Faculty of Agricultural Sciences at the UNSM. The methodologies established by Ríos-Ruiz et al. [31] were used to evaluate the soil properties (Table 2).

Table 2. Physicochemical analysis of the soil under study.

Sample	pH	EC ($\mu\text{s cm}^{-1}$)	OM (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Textural Class
1	7.025	26.35	3.2	10.25	61.77	52	30	18	Sandy loam

EC = electrical conductivity; OM = organic matter; P = phosphorus, and K = potassium. The analyses were performed according to the methodologies described in Ríos-Ruiz et al. [31].

3.2. Inhibition Test

The results of the inhibition test, presented in Figure 1, show the behavior of the rhizobial strain *Ensifer teranga* R1-012-02 in the presence of different phosphate-solubilizing strains. The strain *E. teranga* R1-012-02 (27) exhibited notable growth in the presence of the phosphate-solubilizing strain *Agrobacterium* sp. Sfl-043-09 (15). In contrast, *E. teranga* R1-012-02 did not show any growth when exposed to the phosphate-solubilizing strains *Enterobacter* sp. Sfcm-014-02 (24) and *Enterobacter* sp. Sfcm-054-06 (49). These results suggest a specific interaction between the strains, indicating that *E. teranga* R1-012-02 may be tolerant to *Agrobacterium* sp. Sfl-043-09, but susceptible to the inhibitory effects of *Enterobacter* sp. Sfcm-014-02 (24) and *Enterobacter* sp. Sfcm-054-06.



Figure 1. Inhibition test showing the growth of the rhizobial strain *Ensifer teranga* R1-012-02 (27) in the presence of the phosphate-solubilizing strains *Agrobacterium* sp. Sfl-043-09 (15), *Enterobacter* sp. Sfcm-014-02 (24), and *Enterobacter* sp. Sfcm-054-06 (49). Additionally, strain Sfcv-098-02 (61) also displays inhibitory effects.

The inhibition tests demonstrated that the strains *Enterobacter* sp. Sfcm-014-02 and *Enterobacter* sp. Sfcm-054-06 exhibited a high degree of “in vitro” inhibition against the strain *E. teranga* R1-012-02, effective for *L. leucocephala*; similarly, they were antagonistic toward the strain *Bradyrhizobium glycinis* Rcm-025-01, effective for *C. macrocarpum*. In contrast, the strains *Micrococcus* sp. Sfcm-14-01 and *Agrobacterium* sp. Sfl-043-09 did not show any degree of inhibition against the effective rhizobial strains tested in this study (Table 3).

Table 3. Area of growth inhibition of phosphate-solubilizing bacterial strains on effective rhizobial strains with *Leucaena leucocephala* and *Centrosema macrocarpum*.

Phosphate-Solubilizing Bacterial Strains	Inhibition Area (cm ²) of <i>Ensifer Terangae</i> R1-012-02, Effective with <i>L. leucocephala</i>	Inhibition Area (cm ²) of <i>Bradyrhizobium glycinis</i> Rcm-025-01, Effective with <i>C. macrocarpum</i>
<i>Micrococcus</i> sp. Sfcm-014-01 (PQ215700)	NI	NI
<i>Agrobacterium</i> sp. Sfl-043-09 (PP319609)	NI	NI
<i>Enterobacter</i> sp. Sfcm-014-02 ^T (PP319605)	6.37 ± 0.02	5.78 ± 0.02
<i>Enterobacter</i> sp. Sfcm-054-06 (PP319604)	5.91 ± 0.03	6.36 ± 0.02

NI = no inhibition of growth. The results represent the average of three replicates, along with the standard deviation.

3.3. Bacterial Growth Analysis

A growth curve was developed to determine the number of generations and the generation time of each strain to evaluate the behavior of the studied strains. The results showed that the strain *Micrococcus* sp. Sfcm-14-01 had a generation time of 2.48 h, achieving 15.33 generations. In contrast, *Agrobacterium* sp. Sfl-043-09 reached 14.24 generations with a generation time of 2.25 h. In comparison, the effective rhizobial strains, such as *E. terangae* R1-012-02 and *B. glycinis* Rcm-025-01, demonstrated a higher number of generations, with 20.81 and 20.17, respectively. *E. terangae* R1-012-02 recorded the shortest generation time among all evaluated strains, at 2.11 h (Table 4).

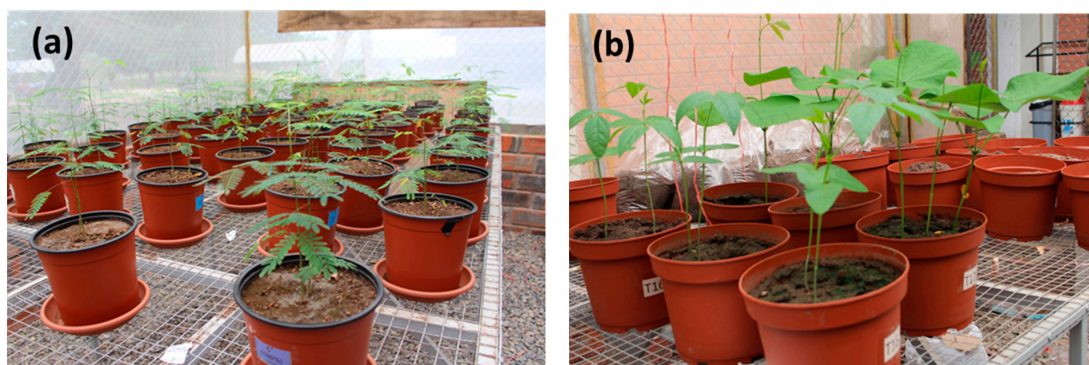
Table 4. Generation times of phosphate-solubilizing bacteria and effective rhizobia selected for co-inoculation tests.

Strains Under Study	Strain Type	Number of Generations	Generation Time (hours)
<i>Micrococcus</i> sp. Sfcm-014-01	PSB	15.33	2.48
<i>Agrobacterium</i> sp. Sfl-043-09	PSB	14.24	2.25
<i>E. terangae</i> R1-012-02	Rhizobium	20.81	2.11
<i>B. glycinis</i> Rcm-025-01	Rhizobium	20.17	4.17

3.4. Evaluation of Co-Inoculation Between PSB, Rhizobia, and Legumes in Semi-Controlled Conditions in a Shade House

3.4.1. Plant Development in Semi-Controlled Conditions in a Shade House

Figure 2 shows the growth of *L. leucocephala* and *C. macrocarpum* under semi-controlled conditions in a mesh house. The plants were incubated for a period of 90 days. The presented images reflect the morphology of the plants, and various parameters, detailed in the Materials and Methods Section, were evaluated.

**Figure 2.** Development of *Leucaena leucocephala* (a) and *Centrosema macrocarpum* (b) under semi-controlled conditions in a shade house. The figures show the plants after a 45-day development period.

3.4.2. Chlorophyll Level in the Plant (SPAD)

Table 5 shows the chlorophyll level, measured in SPAD units, for *L. leucocephala* and *C. macrocarpum* under different co-inoculation treatments with the PSB and rhizobial strains and different levels of phosphate fertilization.

Table 5. Chlorophyll level in *Leucaena leucocephala* and *Centrosema macrocarpum* in response to co-inoculation with phosphate-solubilizing bacterial and rhizobial strains, under different levels of phosphate fertilization (0%, 25%, 50%, and 75%).

Chlorophyll Level in the Plant (SPAD)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	44.05 (±1.16) cdefg	Sfcm-014-01 + Rcm-025-01	35.83 (±0.48) h
Sfl-043-09 + Rl-012-02	43.03 (±0.87) efg	Sfl-043-09 + Rcm-025-01	35.60 (±0.60) h
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	44.84 (±0.78) abcdef	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	36.03 (±0.85) h
Rl-012-02	42.06 (±1.46) fg	Rcm-025-01	36.69 (±0.92) gh
Control	41.75 (±1.18) g	Control	36.01 (±1.12) h
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	44.46 (±1.30) bcdefg	Sfcm-014-01 + Rcm-025-01	41.39 (±0.60) abcd
Sfl-043-09 + Rl-012-02	44.56 (±0.26) bcdefg	Sfl-043-09 + Rcm-025-01	38.23 (±0.90) efgh
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	44.21 (±1.50) cdefg	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	37.39 (±1.21) gh
Rl-012-02	43.78 (±0.62) defg	Rcm-025-01	38.16 (±1.48) fgh
Control	41.83 (±1.19) g	Control	39.41 (±0.73) cdefg
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	46.74 (±0.19) abc	Sfcm-014-01 + Rcm-025-01	41.37 (±1.72) abcd
Sfl-043-09 + Rl-012-02	45.26 (±0.91) abcde	Sfl-043-09 + Rcm-025-01	39.75 (±0.67) bcdefg
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	47.47 (±1.24) a	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	41.29 (±0.64) abcde
Rl-012-02	44.81 (±1.48) abcdef	Rcm-025-01	38.56 (±0.95) cdefgh
Control	45.94 (±0.60) abcd	Control	39.58 (±1.42) cdefg
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	46.68 (±1.04) abc	Sfcm-014-01 + Rcm-025-01	42.76 (±1.37) ab
Sfl-043-09 + Rl-012-02	47.10 (±0.49) ab	Sfl-043-09 + Rcm-025-01	43.42 (±1.22) a
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	47.14 (±0.26) ab	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	42.77 (±1.07) ab
Rl-012-02	46.20 (±1.19) abcd	Rcm-025-01	40.87 (±1.38) abcdef
Control	46.42 (±0.40) abcd	Control	41.14 (±0.69) abcdef
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	47.13 (±0.84) ab	Control	41.77 (±1.29) abc
<i>p</i> -Value	0.0004 **	<i>p</i> -Value	0.0001 **
CV (%)	3.81	CV (%)	4.71

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at $p < 0.05$. CV = coefficient of variation.

The highest chlorophyll levels for *L. leucocephala* were observed in the treatments with the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *Ensifer teranga* Rl-012-02 at a 50% phosphorus (P) dose, with an average value of 47.47 ± 1.24 SPAD. This was closely followed by the 100% NPK fertilization treatment (47.13 ± 0.84 SPAD) and the co-inoculation treatment with the same strains at a 75% P dose (47.14 ± 0.26 SPAD). These values were significantly higher than those of the treatments without phosphate fertilization (0% P), where the lowest chlorophyll level was observed, particularly in the non-fertilized treatment (41.75 ± 1.18 SPAD).

The highest chlorophyll levels for *C. macrocarpum* were recorded in the treatment with *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01 at a 75% P dose, with an average value of 43.42 ± 1.22 SPAD. This treatment showed statistically equivalent values to the other treatments, such as co-inoculation with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 at a 75% P dose and 100% NPK. In contrast, treat-

ments without phosphate fertilization (0% P) showed the lowest chlorophyll levels, with the lowest being for the treatment with *Micrococcus* sp. Sfcm-14-01 and *B. glycinis* Rcm-025-01 (35.83 ± 0.48 SPAD).

3.4.3. Plant Height (cm)

Table 6 shows the height of *L. leucocephala* and *C. macrocarpum* in response to co-inoculation with PSB and rhizobial strains under different levels of phosphate fertilization.

Table 6. Plant height of *Leucaena leucocephala* and *Centrosema macrocarpum* in response to co-inoculation with phosphate-solubilizing bacterial and rhizobial strains, under different levels of phosphate fertilization (0%, 25%, 50%, and 75%).

Plant Height (cm)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	22.17 (±0.83) cdef	Sfcm-014-01 + Rcm-025-01	260.00 (±3.46) def
Sfl-043-09 + Rl-012-02	20.67 (±0.33) efghi	Sfl-043-09 + Rcm-025-01	270.00 (±2.00) cde
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	21.83 (±0.44) cdef	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	223.00 (±7.94) g
Rl-012-02	23.83 (±0.93) bc	Rcm-025-01	270.33 (±16.33) cde
Control	20.33 (±0.60) fghi	Control	248.50 (±13.55) efg
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	22.33 (±0.44) bcdef	Sfcm-014-01 + Rcm-025-01	265.67 (±4.26) def
Sfl-043-09 + Rl-012-02	21.67 (±0.67) cdefg	Sfl-043-09 + Rcm-025-01	267.67 (±5.17) cde
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	18.50 (±0.29) hi	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	264.33 (±13.09) def
Rl-012-02	21.33 (±0.73) cdefg	Rcm-025-01	248.33 (±7.26) efg
Control	21.00 (±1.53) defgh	Control	174.33 (±12.44) h
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	22.50 (±1.32) bcdef	Sfcm-014-01 + Rcm-025-01	280.00 (±2.31) bcd
Sfl-043-09 + Rl-012-02	21.50 (±0.58) cdefg	Sfl-043-09 + Rcm-025-01	318.00 (±9.17) a
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	20.50 (±0.50) efghi	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	296.67 (±6.01) abc
Rl-012-02	18.33 (±0.33) i	Rcm-025-01	316.67 (±8.82) a
Control	19.17 (±1.64) ghi	Control	288.67 (±15.93) abcd
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	23.33 (±1.09) bcd	Sfcm-014-01 + Rcm-025-01	227.00 (±14.57) g
Sfl-043-09 + Rl-012-02	27.17 (±1.20) a	Sfl-043-09 + Rcm-025-01	236.67 (±8.74) fg
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	27.00 (±1.00) a	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	274.67 (±7.42) cde
Rl-012-02	20.67 (±1.20) efghi	Rcm-025-01	271.67 (±1.67) cde
Control	24.83 (±0.60) ab	Control	263.33 (±17.17) def
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	22.92 (±0.58) bcde	Control	304.67 (±13.48) ab
<i>p</i> -Value	0.0001 **	<i>p</i> -Value	0.0001 **
CV (%)	7.03	CV (%)	6.73

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at *p* < 0.05. CV = coefficient of variation.

Significant differences in plant height were observed for *L. leucocephala* based on the inoculant combinations and phosphate fertilization levels. With 0 kg P ha⁻¹ (0% P), the plant height ranged from 20.33 cm (±0.60) without inoculation to 23.83 cm (±0.93) with the strain *E. teranga* Rl-012-02, indicating the inoculation's positive impact. As the phosphate fertilization increased, so did the plant height, reaching a maximum of 27.17 cm (±1.20) with the co-inoculation of *Agrobacterium* sp. Sfl-043-09 and *E. teranga* Rl-012-02 at 66.36 kg P ha⁻¹ (75% P).

Significant differences in height were also observed for *C. macrocarpum* under different treatments. The height with 0 kg P ha⁻¹ ranged between 223.00 cm (±7.94) with the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *Bradyrhizobium glycinis* Rcm-025-01, and 270.33 cm (±16.33) with *B. glycinis* Rcm-025-01. The maximum height of 318.00 cm (±9.17)

was achieved with the co-inoculation of *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01 at 44.24 kg P ha⁻¹ (50% P).

3.4.4. Dry Biomass of Aerial Part (mg)

Table 7 presents the dry biomass of the aerial part of *L. leucocephala* and *C. macrocarpum* based on the co-inoculation treatments with the PSB and rhizobial strains and different phosphate fertilization doses.

Table 7. Dry biomass of the aerial part of *Leucaena leucocephala* and *Centrosema macrocarpum* in response to co-inoculation with phosphate-solubilizing bacterial and rhizobial strains, under different phosphate fertilization levels (0%, 25%, 50%, and 75%).

Dry Biomass of Aerial Part (mg plant ⁻¹)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	372.16 (±19.92) ij	Sfcm-014-01 + Rcm-025-01	1092.45 (±184.89) ghij
Sfl-043-09 + Rl-012-02	492.38 (±64.45) efgh	Sfl-043-09 + Rcm-025-01	1209.88 (±68.74) fghi
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	207.19 (±18.93) k	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	851.70 (±70.73) j
Rl-012-02	293.38 (±16.20) jk	Rcm-025-01	896.79 (±139.76) ij
Control	376.02 (±11.33) hij	Control	946.46 (±151.55) ij
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	389.44 (±12.12) ghij	Sfcm-014-01 + Rcm-025-01	1525.45 (±39.29) bcde
Sfl-043-09 + Rl-012-02	522.40 (±26.21) def	Sfl-043-09 + Rcm-025-01	1329.71 (±87.84) efg
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	460.86 (±76.14) efghi	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	1502.88 (±71.39) cdef
Rl-012-02	430.51 (±61.54) fghi	Rcm-025-01	972.59 (±84.96) hij
Control	418.33 (±18.59) fghi	Control	1055.45 (±56.02) ghij
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	435.32 (±31.56) fghi	Sfcm-014-01 + Rcm-025-01	1327.98 (±45.48) efg
Sfl-043-09 + Rl-012-02	611.69 (±22.01) bcd	Sfl-043-09 + Rcm-025-01	1672.83 (±84.89) abcd
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	558.40 (±17.37) cde	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	1473.83 (±67.71) def
Rl-012-02	494.81 (±16.56) defg	Rcm-025-01	1761.75 (±124.63) abcd
Control	731.55 (±27.20) a	Control	1522.03 (±52.39) bcdef
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	437.75 (±15.30) fghi	Sfcm-014-01 + Rcm-025-01	1282.41 (±207.92) efgh
Sfl-043-09 + Rl-012-02	671.34 (±67.06) abc	Sfl-043-09 + Rcm-025-01	1808.75 (±173.37) abc
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	771.69 (±10.91) a	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	1772.29 (±180.29) abcd
Rl-012-02	706.08 (±51.71) ab	Rcm-025-01	1835.09 (±55.96) ab
Control	660.63 (±89.26) abc	Control	1704.01 (±45.01) abcd
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	682.66 (±38.40) ab	Control	1981.70 (±16.16) a
<i>p</i> -Value	0.0001 **	<i>p</i> -Value	0.0001 **
CV (%)	14.01	CV (%)	13.56

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at *p* < 0.05. CV = coefficient of variation.

For *L. leucocephala*, the treatment with 44.24 kg P ha⁻¹ (50% P) without microbial inoculation produced the highest dry aerial biomass, reaching 731.55 mg plant⁻¹. Among the co-inoculation treatments, the combination of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* Rl-012-02 at 66.36 kg P ha⁻¹ (75% P) showed the highest increase in dry biomass, with 771.69 mg plant⁻¹. In contrast, the lowest values were recorded with 0 kg P ha⁻¹ (0% P) with the combination of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* Rl-012-02, reaching 207.19 mg plant⁻¹.

For *C. macrocarpum*, the treatment with 100% NPK fertilization produced the highest dry biomass, with 1981.70 mg plant⁻¹. Among the inoculated treatments, *B. glycinis* Rcm-025-01 with 56.16 kg P ha⁻¹ (75% P) showed a notably high dry biomass of 1835.09 mg plant⁻¹. The

lowest values were observed under 0 kg P ha⁻¹ (0% P) with the combination of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01, with 851.70 mg plant⁻¹.

3.4.5. Dry Root Biomass (mg)

Table 8 presents the results of the dry root biomass for *L. leucocephala* and *C. macrocarpum* in response to co-inoculation with the PSB and rhizobial strains and different levels of phosphate fertilization.

Table 8. Dry root biomass and response of *Leucaena leucocephala* and *Centrosema macrocarpum* to co-inoculation with PSB and rhizobial strains under different levels of phosphate fertilization (0%, 25%, 50%, and 75%).

Dry Root Biomass (mg plant ⁻¹)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	348.28 (±16.09) defg	Sfcm-014-01 + Rcm-025-01	225.93 (±79.13) efg
Sfl-043-09 + Rl-012-02	417.06 (±47.43) cde	Sfl-043-09 + Rcm-025-01	277.58 (±37.22) cdef
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	346.29 (±75.85) defg	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	161.53 (±8.15) g
Rl-012-02	336.71 (±28.71) efg	Rcm-025-01	197.33 (±33.87) fg
Control	331.45 (±21.21) efg	Control	235.31 (±11.24) efg
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	279.20 (±4.72) g	Sfcm-014-01 + Rcm-025-01	273.60 (±11.17) def
Sfl-043-09 + Rl-012-02	411.63 (±10.97) cde	Sfl-043-09 + Rcm-025-01	315.27 (±41.01) bcde
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	411.93 (±34.63) cde	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	374.40 (±21.18) abc
Rl-012-02	384.63 (±13.55) cdef	Rcm-025-01	216.07 (±27.74) efg
Control	392.78 (±12.14) cdef	Control	220.28 (±10.26) efg
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	573.81 (±55.25) a	Sfcm-014-01 + Rcm-025-01	290.03 (±26.91) bcdef
Sfl-043-09 + Rl-012-02	560.57 (±17.03) ab	Sfl-043-09 + Rcm-025-01	384.20 (±22.38) ab
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	408.55 (±32.16) cde	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	302.59 (±8.02) bcde
Rl-012-02	303.25 (±17.83) fg	Rcm-025-01	351.21 (±23.11) abcd
Control	377.53 (±11.62) cdef	Control	295.56 (±17.63) bcdef
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	423.18 (±61.43) cde	Sfcm-014-01 + Rcm-025-01	387.55 (±32.68) ab
Sfl-043-09 + Rl-012-02	578.27 (±39.52) a	Sfl-043-09 + Rcm-025-01	421.74 (±44.43) a
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	467.23 (±20.07) bc	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	416.86 (±82.32) a
Rl-012-02	552.23 (±35.52) ab	Rcm-025-01	364.17 (±28.40) abcd
Control	434.28 (±8.62) cd	Control	378.58 (±7.69) ab
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	577.53 (±36.62) a	Control	430.09 (±20.56) a
<i>p</i> -Value	0.0001 **	<i>p</i> -Value	0.0001 **
CV (%)	13.92	CV (%)	19.38

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at *p* < 0.05. CV = coefficient of variation.

For *L. leucocephala*, significant differences in the dry root biomass were observed between the various treatments. The treatment with co-inoculation of *Agrobacterium* sp. Sfl-043-09 and *E. teranga* Rl-012-02 with 66.36 kg of P ha⁻¹ (75% P) produced the highest dry root biomass value, reaching 578.27 mg plant⁻¹. In contrast, the lowest value was recorded with the co-inoculation of *Micrococcus* sp. Sfcm-14-01 and *E. teranga* Rl-012-02 at 22.12 kg of P ha⁻¹ (25% P), with 279.20 mg plant⁻¹.

For *C. macrocarpum*, the highest value of dry root biomass was obtained with the 100% NPK fertilization treatment, reaching 430.09 mg per plant. Treatments with the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 at 56.16 kg of P ha⁻¹ (75% P) (416.86 mg plant⁻¹) and *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01 at the same dose (421.74 mg plant⁻¹) also showed high

values. The treatment with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 at 0 kg of P ha⁻¹ (0% P) exhibited the lowest dry root biomass, with 161.53 mg plant⁻¹.

3.4.6. Root Length (cm)

Table 9 presents the root length of *L. leucocephala* and *C. macrocarpum* in response to co-inoculation with the PSB and rhizobial strains under different levels of phosphate fertilization.

Table 9. Root length in *Leucaena leucocephala* and *Centrosema macrocarpum* in response to co-inoculation with PSB and rhizobial strains under different levels of phosphate fertilization (0%, 25%, 50%, and 75%).

Root Length (cm)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	21.00 (±0.58) efg	Sfcm-014-01 + Rcm-025-01	24.67 (±0.67) efgh
Sfl-043-09 + Rl-012-02	21.67 (±0.33) def	Sfl-043-09 + Rcm-025-01	25.67 (±0.88) defgh
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	18.00 (±0.00) hi	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	23.33 (±0.33) h
Rl-012-02	18.67 (±0.33) hi	Rcm-025-01	24.33 (±0.33) fgh
Control	17.00 (±0.58) i	Control	23.33 (±0.33) h
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	22.67 (±0.33) cde	Sfcm-014-01 + Rcm-025-01	25.00 (±0.00) efgh
Sfl-043-09 + Rl-012-02	23.00 (±0.00) bcd	Sfl-043-09 + Rcm-025-01	26.67 (±0.33) bcde
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	24.33 (±0.33) abc	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	23.00 (±0.00) h
Rl-012-02	21.67 (±1.33) def	Rcm-025-01	23.33 (±0.83) h
Control	17.50 (±0.76) i	Control	23.33 (±1.69) h
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	21.00 (±1.00) efg	Sfcm-014-01 + Rcm-025-01	23.67 (±0.33) gh
Sfl-043-09 + Rl-012-02	24.33 (±0.33) abc	Sfl-043-09 + Rcm-025-01	28.67 (±0.67) ab
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	19.67 (±0.67) gh	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	26.00 (±0.58) cdef
Rl-012-02	23.00 (±0.58) bcd	Rcm-025-01	24.33 (±0.33) fgh
Control	24.00 (±0.58) abc	Control	26.17 (±0.73) cdef
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	24.33 (±0.67) abc	Sfcm-014-01 + Rcm-025-01	24.33 (±0.33) fgh
Sfl-043-09 + Rl-012-02	25.00 (±0.00) a	Sfl-043-09 + Rcm-025-01	29.67 (±0.88) a
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	23.33 (±0.67) abcd	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	27.33 (±1.45) bcd
Rl-012-02	24.67 (±0.33) ab	Rcm-025-01	24.67 (±0.88) efgh
Control	21.17 (±0.73) efg	Control	27.17 (±0.73) bcd
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	20.67 (±0.73) fg	Control	28.00 (±0.58) abc
<i>p</i> -Value	0.0001 **	<i>p</i> -Value	0.0001 **
CV (%)	4.84	CV (%)	5.03

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at *p* < 0.05. CV = coefficient of variation.

Significant differences in root length were observed between the treatments for *L. leucocephala*. The longest roots were recorded with the co-inoculation of *Agrobacterium* sp. Sfl-043-09 and *E. teranga* Rl-012-02 at 66.36 kg of P ha⁻¹ (75% P), reaching 25.00 cm. This result was comparable to other high-phosphorus treatments, such as co-inoculation with *Micrococcus* sp. Sfcm-14-01 and *E. teranga* Rl-012-02 (24.33 cm) and *E. teranga* Rl-012-02 alone (24.67 cm). In contrast, the treatment without phosphate fertilization (0 kg of P ha⁻¹) showed the shortest root lengths, with values as low as 17.00 cm.

Differences in root length were also observed between treatments for *C. macrocarpum*. The greatest length was obtained with the co-inoculation of *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01 at 66.36 kg of P ha⁻¹ (75% P), reaching 29.67 cm. Other treatments with high-phosphate fertilization, such as co-inoculation with *Micrococcus* sp. Sfcm-14-01,

Agrobacterium sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 at 66.36 kg of P ha⁻¹ (75% P), also showed elevated root lengths (27.33 cm). Similar to *L. leucocephala*, the shortest root lengths in *C. macrocarpum* were observed in treatments without phosphate fertilization, with a length of 23.33 cm.

3.4.7. Leaf Phosphorus (P) (ppm)

Table 10 shows the leaf phosphorus levels (ppm) in *L. leucocephala* and *C. macrocarpum* in response to co-inoculation with the PSB and rhizobial strains under different levels of phosphate fertilization.

Table 10. Leaf phosphorus levels in *Leucaena leucocephala* and *Centrosema macrocarpum* in response to co-inoculation with PSB and rhizobial strains under different levels of phosphate fertilization (0%, 25%, 50%, and 75%).

Leaf Phosphorus (ppm)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + RI-012-02	173.23 (±4.16) abcdefgh	Sfcm-014-01 + Rcm-025-01	240.00 (±40.70) hij
Sfl-043-09 + RI-012-02	148.07 (±4.42) efghi	Sfl-043-09 + Rcm-025-01	245.33 (±22.02) hij
Sfcm-014-01 + Sfl-043-09 + RI-012-02	164.07 (±7.51) cdefgh	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	243.33 (±2.96) hij
RI-012-02	143.40 (±7.23) abcde	Rcm-025-01	254.33 (±13.74) bcde
Control	121.70(±0.56) i	Control	213.00 (±17.01) j
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + RI-012-02	178.73 (±1.89) abcde	Sfcm-014-01 + Rcm-025-01	260.67 (±13.20) fghij
Sfl-043-09 + RI-012-02	150.00 (±17.73) defghi	Sfl-043-09 + Rcm-025-01	291.00 (±2.65) cdefgh
Sfcm-014-01 + Sfl-043-09 + RI-012-02	184.36 (±8.47) abc	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	303.00 (±18.77) bcdefg
RI-012-02	176.20 (±18.02) abcdef	Rcm-025-01	270.33 (±6.69) ghij
Control	142.27 (±20.52) hi	Control	226.67 (±9.39) ij
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + RI-012-02	174.40 (±10.27) abcdefgh	Sfcm-014-01 + Rcm-025-01	325.33 (±11.61) abcd
Sfl-043-09 + RI-012-02	181.00 (±1.73) abcd	Sfl-043-09 + Rcm-025-01	317.67 (±14.66) abcde
Sfcm-014-01 + Sfl-043-09 + RI-012-02	197.37 (±8.61) ab	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	312.00 (±6.56) abcdef
RI-012-02	173.80 (±10.41) abcdefgh	Rcm-025-01	272.00 (±17.06) defghi
Control	146.00 (±30.19) fghi	Control	253.00 (±4.04) ghij
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + RI-012-02	174.57 (±2.38) abcdefg	Sfcm-014-01 + Rcm-025-01	330.00 (±15.28) abc
Sfl-043-09 + RI-012-02	177.50 (±11.19) abcdef	Sfl-043-09 + Rcm-025-01	334.33 (±28.20) abc
Sfcm-014-01 + Sfl-043-09 + RI-012-02	186.67 (±10.48) abc	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	353.67 (±13.68) ab
RI-012-02	178.67 (±7.26) abcde	Rcm-025-01	317.67 (±26.74) abcde
Control	168.33 (±11.26) bcdefgh	Control	309.33 (±25.57) bcdef
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	201.67 (±12.33) a	Control	365.33 (±32.84) a
<i>p</i> -Value	0.0001 **	<i>p</i> -Value	0.0001 **
CV (%)	12.32	CV (%)	11.50

Strains: Sfcm-014-01 = *Micrococcus* sp.; RI-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at *p* < 0.05. CV = coefficient of variation.

The leaf phosphorus levels in *L. leucocephala* significantly varied between the different treatments. The highest leaf phosphorus content was observed with 100% NPK fertilization, reaching 201.67 ppm. Treatments with high-phosphate fertilization (44.24 kg of P ha⁻¹; 50% P) also showed elevated levels, notably the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* RI-012-02, with 197.37 ppm. In contrast, the treatment without fertilization had the lowest leaf phosphorus content, at 121.70 ppm, suggesting a positive relationship between the phosphate fertilization level and phosphorus content in the leaves.

Significant differences in the leaf phosphorus levels in *C. macrocarpum* were also observed between treatments. The highest content was recorded with 100% NPK fertilization, reaching 365.33 ppm. The other treatments with high-phosphorus doses, such as the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 at 66.36 kg of P ha⁻¹ (75% P), showed elevated leaf phosphorus levels, at 353.67 ppm. Similar to *L. leucocephala*, the treatment without fertilization showed one of the lowest leaf phosphorus levels, at 213.00 ppm.

3.4.8. Phosphorus (P) in Soil (ppm)

Table 11 presents the phosphorus levels in the soil (ppm) accumulated in *L. leucocephala* and *C. macrocarpum* in response to co-inoculation with PSB and rhizobial strains under different levels of phosphate fertilization.

Table 11. Soil phosphorus levels in response to co-inoculation with PSB and rhizobial strains in *Leucaena leucocephala* and *Centrosema macrocarpum* under different levels phosphate fertilization (0%, 25%, 50%, and 75%).

Soil Phosphorus (ppm)			
<i>L. leucocephala</i>		<i>C. macrocarpum</i>	
0 kg P ha ⁻¹ (0% P)		0 kg P ha ⁻¹ (0% P)	
Sfcm-014-01 + Rl-012-02	5.49 (±0.02) h	Sfcm-014-01 + Rcm-025-01	3.74 (±0.01) l
Sfl-043-09 + Rl-012-02	5.82 (±0.07) h	Sfl-043-09 + Rcm-025-01	4.41 (±0.04) h
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	5.58 (±0.01) h	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	5.41 (±0.01) fg
Rl-012-02	6.09 (±0.03) g	Rcm-025-01	4.58 (±0.00) gh
Control	5.07 (±0.00) h	Control	2.26 (±0.06) j
22.12 kg P ha ⁻¹ (25% P)		18.72 kg P ha ⁻¹ (25% P)	
Sfcm-014-01 + Rl-012-02	6.82 (±0.05) g	Sfcm-014-01 + Rcm-025-01	5.57 (±0.04) f
Sfl-043-09 + Rl-012-02	7.27 (±0.02) g	Sfl-043-09 + Rcm-025-01	5.41 (±0.09) efg
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	7.57 (±0.09) fg	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	5.74 (±0.00) f
Rl-012-02	6.91 (±0.07) g	Rcm-025-01	4.91 (±0.02) g
Control	6.90 (±0.03) g	Control	3.74 (±0.00) l
44.24 kg P ha ⁻¹ (50% P)		37.44 kg P ha ⁻¹ (50% P)	
Sfcm-014-01 + Rl-012-02	7.82 (±0.14) defg	Sfcm-014-01 + Rcm-025-01	6.91 (±0.08) abcd
Sfl-043-09 + Rl-012-02	8.74 (±0.06) cde	Sfl-043-09 + Rcm-025-01	6.57 (±0.01) d
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	9.15 (±0.13) bc	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	5.80 (±0.04) ef
Rl-012-02	7.15 (±0.01) g	Rcm-025-01	5.07 (±0.00) g
Control	8.55 (±0.06) cde	Control	6.24 (±0.00) de
66.36 kg P ha ⁻¹ (75% P)		56.16 kg P ha ⁻¹ (75% P)	
Sfcm-014-01 + Rl-012-02	8.40 (±0.04) cef	Sfcm-014-01 + Rcm-025-01	7.24 (±0.02) b
Sfl-043-09 + Rl-012-02	10.23 (±0.11) b	Sfl-043-09 + Rcm-025-01	7.49 (±0.03) ab
Sfcm-014-01 + Sfl-043-09 + Rl-012-02	13.73 (±0.02) a	Sfcm-014-01 + Sfl-043-09 + Rcm-025-01	7.69 (±0.03) a
Rl-012-02	8.24 (±0.03) cef	Rcm-025-01	6.24 (±0.00) de
Control	8.69 (±0.02) ce	Control	6.38 (±0.03) d
119.76 kg N ha ⁻¹ + 88.48 kg P ha ⁻¹ + 99.54 kg K ha ⁻¹ (100% NPK)		119.76 kg N ha ⁻¹ + 74.89 kg P ha ⁻¹ + 59.88 kg K ha ⁻¹ (100% NPK)	
Control	9.15(±0.00) bcd	Control	6.94 (±0.03) c
p-Value	0.0001 **	p-Value	0.0001 **
CV (%)	1.38	CV (%)	1.12

Strains: Sfcm-014-01 = *Micrococcus* sp.; Rl-012-02 = *E. teranga*; Sfl-043-09 = *Agrobacterium* sp.; Rcm-025-01 = *B. glycinis*. Control = strain-free treatment. The statistical differences between means (n = 3) are represented by different letters, according to the Fisher LSD test. ** = highly significant at p < 0.05. CV = coefficient of variation.

The soil phosphorus levels significantly varied by treatment in *L. leucocephala*. The highest level was observed with the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* Rl-012-02 under 66.36 kg P ha⁻¹ (75% P) fertilization, reaching 13.73 ppm, a notable increase compared with the no-fertilization treatment (5.07 ppm). Other high-phosphorus fertilization treatments, such as the application of 44.24 kg P ha⁻¹ (50% P), also showed elevated soil phosphorus levels, particularly with the co-inoculation of *Micrococcus*

sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* R1-012-02 (9.15 ppm). These results indicate a growing trend in soil phosphorus levels with increased phosphate fertilization.

The soil phosphorus levels also showed significant variations between treatments in *C. macrocarpum*. The highest level was recorded with the co-inoculation of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 under 56.16 kg P ha⁻¹ (75% P) fertilization, reaching 7.69 ppm. This value was considerably higher than that with the no-fertilization treatment (2.26 ppm), suggesting a positive response of soil phosphorus to phosphate fertilization. Although the overall soil phosphorus levels in *C. macrocarpum* were lower than those observed in *L. leucocephala*, the pattern of increase with fertilization was consistent in both species.

4. Discussion

Strains that exhibited negative effects in the inhibition test were identified and, thus, excluded from future co-inoculation studies. In contrast, strains that showed no degree of inhibition against effective rhizobia (Table 3) were selected for further testing under semi-controlled conditions. The negative interaction observed between native *Enterobacter* strains and rhizobia represents the first report of this kind for strains isolated from tropical soils in the Amazon. Studies on the inhibition using non-rhizobial bacteria associated with soybean nodules have shown that, although they do not inhibit rhizobia, they do inhibit plant pathogens through the production of bacterial metabolites [32]. Understanding these interactions is crucial; so, it is recommended that compatibility tests be conducted between the strains. Although synergistic or antagonistic relationships between microorganisms have been under-documented, there is growing interest in using various strains to provide protection against pathogens and promote plant growth [11,33–35].

The exponential or logarithmic phase is crucial for bacterial reproduction, as it is the period during which microorganisms multiply exponentially and their population grows with each generation cycle under optimal conditions [36]. Knowledge of this process is essential for inoculant formulation, as inoculants should be applied before bacteria reach the stationary phase when the growth rate starts to decline [37]. This study provides, for the first time, data on the generation times of the investigated bacterial species (Table 4). This information is fundamental for optimizing the production and effectiveness of inoculants, ensuring that the bacteria are in their most active phase at the time of application.

The results presented in Tables 5–11 show that co-inoculation with PSB and rhizobial strains significantly influenced the growth of *L. leucocephala* and *C. macrocarpum*. Treatments involving strains of *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, *E. teranga* R1-012-02, and *B. glycinis* Rcm-025-01 demonstrated significant improvements in the evaluated parameters. For the chlorophyll content, applying 75% phosphatic fertilization resulted in a 5.13% increase in *L. leucocephala* when interacting with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* R1-012-02. For *C. macrocarpum*, a 21.92% increase was observed in symbiosis with *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01 compared with the no-fertilization treatment, suggesting a significant improvement in the photosynthetic capacity of both species. Regarding plant height, the 75% phosphatic fertilization treatment resulted in a 31.4% increase in *L. leucocephala* combined with *Agrobacterium* sp. Sfl-043-09 and *E. teranga* R1-012-02, and a 23.17% increase in *C. macrocarpum* in symbiosis with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01, compared with the no-fertilization treatment. Additionally, concerning aerial biomass, using 75% phosphatic fertilization showed increases of 267.99% for *L. leucocephala* in combination with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. teranga* R1-012-02, and 104.63% for *C. macrocarpum* in symbiosis with *B. glycinis* Rcm-025-01, relative to the no-fertilization treatment. Finally, regarding root dry biomass, a 38.6% increase was recorded for *L. leucocephala* with the combination of *Agrobacterium* sp. Sfl-043-09 and *E. teranga* R1-012-02, as well as a 51.95% increase for *C. macrocarpum* in symbiosis with *Agrobacterium* sp. Sfl-043-09 and *B. glycinis* Rcm-025-01, compared with the no-fertilization treatment. These results highlight the positive impact of co-inoculation on the growth and development of both plant species, corroborating its effectiveness in optimizing crop performance.

Similar results were reported by Elkoca et al. [38], who evaluated single, double, and triple inoculations with *Rhizobium*, *Bacillus subtilis* strain OSU-142 (nitrogen fixer), and *Bacillus megaterium* strain M3 (phosphate solubilizer) in *Phaseolus vulgaris*. They observed an increase in chlorophyll content between 34.1% and 59.3% in the inoculated treatments. Other studies have also shown improvements in plant height and biomass. Argaw et al. [39] reported an increase of up to 36.98% in the aerial biomass of *Glycine max*, while Singh et al. [40] found similar results in *Cicer arietinum*. Similarly, Shome et al. [16] demonstrated that strains of *Rhizobium japonicum* and *Pseudomonas striata* improved soil nutrient availability and soybean growth, yield, and quality. Likewise, Cubillos-Hinojosa et al. [41] reported an increase in both aerial and root dry biomass in *L. leucocephala* plants inoculated with rhizobia and *Azospirillum brasilense*.

Those and this study's results demonstrate that the co-inoculation of BSF and rhizobia can enhance plant growth by increasing the availability of essential nutrients, such as phosphorus and nitrogen [18,42,43]. Furthermore, it has been documented that co-inoculation promotes BNF efficiency and improves phosphorus absorption, two critical factors for optimal legume growth [44,45].

Tables 10 and 11 show a significant and positive correlation between the foliar phosphorus content, phosphatic fertilization, and soil phosphorus availability in response to *L. leucocephala* and *C. macrocarpum*. Co-inoculation with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. terangae* R1-012-02 promoted an increase in foliar phosphorus for both species, likely due to the mobilization and efficient use of phosphorus facilitated by the synergistic interaction of the inoculated microorganisms.

The treatment with 75% phosphatic fertilization resulted in a 13.76% increase in the foliar phosphorus content in *L. leucocephala* when interacting with these microorganisms. For *C. macrocarpum*, a 45.4% increase was observed in symbiosis with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01, compared with the no-fertilization treatment. Previous studies align with these results: Elkoca et al. [38] reported a 30.5% increase in phosphorus accumulation in the leaves of *Phaseolus vulgaris* co-inoculated with *Rhizobium*, *Bacillus subtilis*, and *B. megaterium*; Argaw et al. [39] reported a 66.9% increase in *Glycine max* co-inoculated with *Bradyrhizobium japonicum* and *Pseudomonas spp.*; and Qureshi et al. [46] detected a 25.75% increase in *Vigna mungo* inoculated with *Rhizobium* sp. and *Bacillus* sp. Shome et al. [16] also demonstrated that *R. japonicum* increased the soil nitrogen content, while *Pseudomonas striata* improved phosphorus availability.

The highest soil phosphorus levels were obtained with 75% of the recommended fertilization levels, increasing the phosphorus availability by 146.45% for *L. leucocephala* and 42.12% for *C. macrocarpum*, compared with the no-fertilization treatment. Comparing the bacterial inoculant treatments with no inoculant and no phosphatic fertilization treatments showed marked differences. In *L. leucocephala*, the interaction with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. terangae* R1-012-02 resulted in a 170.65% increase; meanwhile, in *C. macrocarpum*, symbiosis with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *B. glycinis* Rcm-025-01 produced a 240.44% increase.

These results reinforce that phosphorus availability is a limiting factor in soils with a low phosphorus content, as noted in the scientific literature. Phosphorus is often found in forms inaccessible to plants in tropical soils, affecting their growth and development [47,48]. Furthermore, phosphatic fertilization has been shown to improve BNF efficiency by enhancing the activity of rhizobia and other symbiotic microorganisms [49].

Tables 5–11 show that specific combinations of bacterial strains had differentiated effects on phosphorus solubilization and BNF. Co-inoculation with *Micrococcus* sp. Sfcm-14-01, *Agrobacterium* sp. Sfl-043-09, and *E. terangae* R1-012-02 produced the highest levels of available phosphorus in the soil and significantly greater plant growth than the other combinations, highlighting the importance of precise strain selection to maximize benefits in nutrient availability and plant development. Singh et al. [40] also reported that the co-inoculation of two PSB (*Pantoea cypripedii* PS1 and *Enterobacter aerogenes* PS16) with *Rhizobium ciceri* in *Cicer arietinum* increased the soil phosphorus content by 73.56% compared with uninoculated controls. Similarly, Benjelloun et al. [50] demonstrated that the combined inoculation of chickpeas with rhizobia and PSB

significantly improved the biomass production, grain yield, and nitrogen and phosphorus contents, surpassing both the single inoculation and N and P fertilization.

Recent studies confirm that the synergistic interaction between PSB and rhizobia increases phosphorus availability and optimizes BNF efficiency, leading to more efficient and sustainable plant growth [35,51,52]. This improvement in phosphorus availability and nitrogen fixation reduces the need for synthetic phosphatic fertilizers, whose excessive use can cause environmental problems such as eutrophication [5]. Additionally, promoting rhizobial–legume symbiosis via co-inoculation can restore fertility in degraded soils, especially in tropical agricultural systems [53]. Adopting these biotechnological practices could increase crop productivity and the sustainability of agricultural systems in the long term, significantly contributing to food security in developing regions [54,55].

However, it is important to highlight that certain phosphate-solubilizing bacteria, such as those belonging to the genera *Micrococcus* sp. and *Agrobacterium* sp., can also have pathogenic effects on animals and plants. Although *Micrococcus* sp. is generally considered non-pathogenic, some species, such as *Micrococcus luteus*, can cause opportunistic infections in immunocompromised individuals, affecting the skin, urinary tract, or respiratory system [56]. On the other hand, *Agrobacterium* sp., such as *Agrobacterium tumefaciens*, is known to cause the “crown gall” disease in dicotyledonous plants, where the bacteria introduce their DNA into the plant cells, forming tumors [13,57]. Despite these pathogenic effects, both bacteria also play a crucial role as plant growth promoters in agricultural applications.

The molecular analysis of the 16S ribosomal gene revealed that the four identified calcium PSB strains belong to the genera *Micrococcus*, *Agrobacterium*, and *Enterobacter*. *Micrococcus* sp. has demonstrated an outstanding phosphate solubilization capability under various environmental conditions [58–60]. However, its effectiveness in specifically solubilizing calcium phosphate has not been fully documented. Meanwhile, *Agrobacterium* sp. has been recognized for its ability to solubilize phosphate in agricultural systems [61,62] and interact with different types of substrates, which makes it a promising option for phosphate-deficient soils. The *Enterobacter* strains have been documented to have a high potential for phosphate solubilization in various environments [63,64]. These strains are effective in improving nutrient availability for plants in agricultural soils. Among the four strains analyzed, *Micrococcus* sp. Sfcm-14-01 (PQ215700) and *Agrobacterium* sp. Sfl-043-09 (PP319609) demonstrated a notable calcium phosphate solubilization capability. Additionally, *Enterobacter* sp. Sfcm-014-02 (PP319605) and *Enterobacter* sp. Sfcm-054-06 (PP319604) also showed high calcium phosphate solubilization activity and were especially effective in releasing this phosphate under specific cultivation conditions.

5. Conclusions

The results of this research demonstrate that the co-inoculation of PSB and rhizobia has a significant positive impact on the growth and development of *Leucaena leucocephala* and *Centrosema macrocarpum*, improving key agronomic parameters such as chlorophyll content, plant height, and aerial and root biomass. Specifically, the combination of strains such as *Micrococcus* sp., *Agrobacterium* sp., and *Ensifer teranga* with 75% phosphatic fertilization increased aerial biomass by up to 267.99% in *L. leucocephala* and 104.63% in *C. macrocarpum*, while the foliar phosphorus content increased by 13.76% and 45.4%, respectively. Moreover, the soil phosphorus availability increased by up to 170.65% in *L. leucocephala* and 240.44% in *C. macrocarpum* compared to unfertilized treatments. These results confirm that co-inoculation enhances phosphorus solubilization and biological nitrogen fixation efficiency, two critical factors for optimal legume growth in low-fertility soils. Additionally, negative interactions between *Enterobacter* strains and rhizobia were identified, highlighting the importance of conducting compatibility tests before application. The improvement in nutrient availability and plant growth underscores the potential of this biotechnological strategy to increase productivity in tropical soils, particularly in regions like the Amazon. However, despite the promising results, this study presents some limitations, as it was conducted under controlled conditions that do not fully reflect the complexity of natural

agroforestry systems, where environmental factors can influence the effectiveness of microbial consortia. It is important that future studies conduct long-term field trials, address the variability of microorganisms in different soil types and climates, and further explore microbial interactions to develop more stable consortia.

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Data Availability Statement: The molecular datasets generated during the current study are available in the NCBI GenBank, with accession numbers PQ215700, PP319609, PP319605, PP319604, PQ345348, and PQ356800. Other datasets used during the present study are available from the corresponding author upon reasonable request.

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