


DOI: <https://doi.org/10.17921/1415-6938.2026v30n1p96-114>


Agronomic Efficiency of Co-Inoculation with *Azospirillum brasilense* and *Pseudomonas Fluorescens* in Corn and Soybean Under Reduced Phosphate Fertilization

Eficiência Agronômica da Co-Inoculação com *Azospirillum brasilense* e *Pseudomonas Fluorescens* em Milho e Soja Sob Redução da Adubação Fosfatada


Received: January, 22, 2026

Accepted: March, 19, 2026

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Abstract

The low availability of phosphorus (P) in tropical soils, resulting from fixation by iron and aluminum oxides, constitutes a major limiting factor for soybean and corn productivity. Plant growth-promoting bacteria represent a promising strategy to enhance nutrient use efficiency in cropping systems. This study evaluated the agronomic efficiency of co-inoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* under a 25% reduction in phosphate fertilization for soybean and corn crops. Field experiments were conducted during the 2019/2020 growing season at four locations in Southern Brazil, using a randomized complete block design. Three treatments without inoculation were applied with 0%, 75%, and 100% of the recommended P₂O₅ dose (P0-NI, P75-NI, and P100-NI, respectively), one with inoculation of the commercial product BiomaPhos[®] and 75% P₂O₅ (P75-BiomaPhos), and one with co-inoculation of *A. brasilense* and *P. fluorescens* and 75% P₂O₅ (P75-Biofree). P75-Biofree maintained grain yields for both soybean (4,250 kg ha⁻¹) and corn (14,013 kg ha⁻¹) at levels statistically equivalent to the full phosphate fertilization treatment (P100-NI), while exceeding the absolute control (P0-NI) by 10.4% and 11.5%, respectively. The yield gains were primarily attributed to improved grain filling in soybean and increased kernel number per ear in corn, without significantly altering tissue phosphorus concentrations. This pattern indicates enhanced internal phosphorus use efficiency mediated by bacterial inoculation. We conclude that co-inoculation with *A. brasilense* and *P. fluorescens* represents an effective agricultural technology, enabling a 25% reduction in mineral phosphate fertilizer application without compromising grain yield, thereby promoting more sustainable and economically viable agricultural systems.

Keywords: Plant Growth-Promoting Bacteria. Phosphorus Use Efficiency. Sustainable Intensification. Nutrient Management. Tropical Soils.

Resumo

A baixa disponibilidade de fósforo (P) em solos tropicais, devido à sua fixação por óxidos de ferro e alumínio, limita a produtividade de soja e milho. Bactérias promotoras de crescimento de plantas representam uma estratégia para aumentar a eficiência do uso de nutrientes. Este estudo avaliou a eficiência agrônômica da co-inoculação com *Azospirillum brasilense* e *Pseudomonas fluorescens* sob redução de 25% na adubação fosfatada para as culturas de soja e milho. Experimentos de campo foram conduzidos na safra 2019/2020 em quatro locais no Sul do Brasil, em delineamento de blocos casualizados. Foram aplicados três tratamentos sem inoculação com 0%, 75% e 100% da dose recomendada de P₂O₅ (P0-NI, P75-NI e P100-NI, respectivamente), um com inoculação do produto comercial BiomaPhos® e 75% de P₂O₅ (P75-BiomaPhos), e um com co-inoculação de *A. brasilense* e *P. fluorescens* e 75% de P₂O₅ (P75-Biofree). P75-Biofree manteve a produtividade de grãos de soja (4.250 kg ha⁻¹) e milho (14.013 kg ha⁻¹) estatisticamente iguais ao tratamento com 100% de P (P100-NI), superando a testemunha absoluta (P0-NI) em 10,4% e 11,5%, respectivamente. Os ganhos de produtividade foram atribuídos principalmente ao enchimento de grãos na soja e ao maior número de grãos por espiga no milho, sem alterar significativamente as concentrações de P nos tecidos, indicando maior eficiência interna de uso de P. Conclui-se que a co-inoculação com *A. brasilense* e *P. fluorescens* é uma tecnologia eficaz, permitindo uma redução de 25% no fertilizante fosfatado mineral sem comprometer o rendimento, promovendo assim um sistema agrícola mais sustentável e econômico.

Palavras-chave: Bactérias Promotoras de Crescimento de Plantas. Eficiência no Uso de Fósforo. Intensificação Sustentável. Manejo de Nutrientes. Solos Tropicais.

1 Introduction

Soybean (*Glycine max* L.) and corn (*Zea mays* L.) are commodities of global economic and food security importance, widely cultivated in Brazil (FAOStat, 2025). One of the reasons for soybean's great success lies in its ability to associate with symbiotic microorganisms of the genus *Bradyrhizobium*, which perform biological nitrogen fixation, supplying a large part of the crop's nitrogen demand—fixing 80 to 300 kg N ha⁻¹, representing substantial cost savings of R\$ 464.80 to R\$ 1,743.00 per hectare (considering an average cost of R\$ 5.81 per kg of N) (Banco Central do Brasil, 2025; Cassán; Diaz-Zorita, 2016; Fukami; Cerny; Burland, 2018). Corn, in turn, owes its high productivity to efficient association with soil microorganisms, mainly plant growth-promoting bacteria (PGPB) such as *Azospirillum brasilense*, which act in associative biological nitrogen fixation and phytohormone production, resulting in an average yield increase of 15 to 30 sacks per hectare, potentially representing an additional economic gain of approximately R\$ 1,800.00 per hectare (considering a price of R\$ 60.00 per sack) (Cepea/Esalq, 2025; Galindo *et al.*, 2022; Hungria *et al.*, 2010).

The nutritional efficiency of soybean and corn, especially in acidic tropical soils, is limited by low phosphorus (P) availability. This occurs due to strong adsorption of this nutrient to iron (Fe³⁺) and aluminum (Al³⁺) oxides and hydroxides, resulting in low use efficiency, with only 10–20% of

applied P absorbed by plants (Rodríguez; Fraga, 1999). This scenario highlights the need for strategies that optimize nutrient acquisition and promote greater plant resilience, as it imposes high operational costs and underutilizes the productive potential of agricultural systems (Santos; Nogueira; Hungria, 2019).

The search for tools to increase nutrient use efficiency has thus become a strategic priority (Hungria *et al.*, 2023). In this context, bioinoculants based on PGPB are consolidated not as a marginal alternative, but as a central technology for more efficient and sustainable production (Moreira; Siqueira, 2006). The use of PGPB is a well-established strategy to improve plant health and growth through diverse mechanisms (Lugtenberg; Kamilova, 2009). The forefront of this research involves the use of synergistic microbial consortia, where different bacteria with complementary modes of action work integrally to optimize crop nutrition and development (Santos; Nogueira; Hungria, 2019).

Among bacterial genera with proven efficacy, *Azospirillum* and *Pseudomonas* stand out, acting through distinct and potentially complementary pathways. *Azospirillum brasilense* is recognized for its remarkable capacity for associative biological nitrogen fixation (BNF) and, mainly, for the synthesis of phytohormones such as indole-3-acetic acid (IAA) (Fukami; Cerny; Burland, 2018). This bacterial hormone acts as a potent stimulator of root development, promoting a deeper, more branched, and abundant root system (Cassán; Díaz-Zorita, 2016). This expanded “root architecture” functions as a more efficient exploration platform, allowing the plant to access a greater volume of water and nutrients already present in the soil profile (Fukami; Cerny; Burland, 2018).

Synergistically, *Pseudomonas fluorescens* acts as a highly efficient phosphate solubilizer. Its mechanism relies on the exudation of organic acids (e.g., gluconic and citric acids), which acidify the rhizosphere microenvironment and chelate cations, dissociating phosphorus bound to minerals such as calcium, iron, and aluminum phosphates (Vey *et al.*, 2025). Concurrently, the production of siderophores and enzymes such as ACC deaminase contributes to a more favorable rhizosphere environment, mitigating abiotic stresses and enhancing beneficial microbial activity (Glick, 2012).

The technological premise guiding this work is that co-inoculation of *A. brasilense* and *P. fluorescens* can establish a positive feedback loop in the rhizosphere (Marchão *et al.*, 2025). In this model, the root system expanded by *Azospirillum* provides an enlarged habitat and greater flow of exudates for colonization and action by *Pseudomonas* (Hungria *et al.*, 2013). In turn, the solubilized phosphorus and stress modulation by *Pseudomonas* provide the energy substrate and ideal conditions for greater root growth and *Azospirillum* activity (Zeffa *et al.*, 2020). This functional integration has the potential to elevate plant physiological efficiency, allowing better conversion of resources into productivity (Nascimento *et al.*, 2024).

The practical manifestation of this synergy, however, must be validated under the distinct

Ensaio e Ciência, v.30, n.1, p.96-114, 2026.

edaphoclimatic conditions of Brazil, where soil, climate, and management variability can directly influence inoculant performance (Zeffa *et al.*, 2023). Given the above, the main objective of this study was to evaluate the agronomic efficiency a consortium composed of *Azospirillum brasilense* (Ab-V6) and *Pseudomonas fluorescens* (CCTB) in soybean and corn. Specifically, we sought to verify the technical feasibility of associating this co-inoculation with a 25% reduction in mineral phosphate fertilization, testing the hypothesis that this biological strategy can maintain or increase grain yield, optimizing plant nutrition and physiology across different productive environments.

2 Material and Methods

Field experiments were conducted to validate the efficiency and viability of the bacterial consortium *Azospirillum brasilense* Ab-V6 and *Pseudomonas fluorescens* CCTB, according to protocols imposed by the Ministry of Agriculture, Livestock, and Supply/Ministério da Agricultura (MAPA) for registration of microbial-based plant growth promoter products, following IN SDA 13 of 25/03/2011 (Brasil, 2011), IN SDA 25 of 28/07/2009 (Brasil, 2009), and IN SDA 53 of 24/10/2013 (Brasil, 2013). The development and quality assurance of microbial inoculants are fundamental to the success of the technology under field conditions (Bashan *et al.*, 2014).

Four locations with distinct edaphoclimatic characteristics were selected (Table 1 and Table 2). Experiments were conducted during the 2019/2020 cropping season.

Table 1 – Description of locations and management practices in trials evaluating the agronomic efficiency of the bacterial consortium *Azospirillum brasilense* and *Pseudomonas fluorescens* in soybean (*Glycine max* L.) across four distinct edaphoclimatic sites

Description	Location			
	Candói (PR)	Guarapuava (PR)	Lapa (PR)	Sertão (RS)
Previous summer crop	Soybean	Soybean	Soybean	Soybean
Previous winter crop	Oat	Oat	Oat	Ryegrass
Soybean cultivar	TMG 7062	TMG 7062	TMG 7062	TMG 7062
Corn cultivar	DKB 345 PRO3	DKB 345 PRO3	DKB 345 PRO3	DKB 345 PRO3
	Geographic Location			
Latitude	25°31'56,9" S	25°22'59" S	25°50'52,3" S	28°01'37" S
Longitude	51°48'0,8" W	51°33'14,5" W	49°39'8,4" W	52°15'52" W
Altitude (m)	920	995	855	720
	Calendar			
	Soybean			
Seeding	11/21/2019	11/08/2019	10/31/2019	11/16/2019
R1	01/20/2020	01/07/2020	01/04/2020	01/15/2020
Harvest	04/04/2020	03/22/2020	03/14/2020	03/30/2020
	Corn			
Seeding	09/27/2019	10/18/2019	10/03/2019	10/09/2019
V4-V6	10/22/2019	11/11/2019	11/30/2019	11/16/2019
Harvest	04/10/2020	04/28/2020	04/15/2020	04/20/2020

Source: research data.

Table 1 – Physical and chemical soil characterization before experiment establishment at the four evaluated sites

Description	Candói (PR)	Guarapuava (PR)	Lapa (PR)	Sertão (RS)
Soil Physical Characterization				
Clay (g/kg)	550	590	220	560
Silt (g/kg)	290	290	310	270
Sand (g/kg)	160	120	470	170
Textural class	Clayey	Clayey	Medium	Clayey
Soil Chemical Characterization				
pH (CaCl ₂)	5.25	5.03	5.26	4.21
SOM (g dm ⁻³)	26.51	46.55	21.75	45.94
P-Mehlich (mg dm ⁻³)	11.9	5.82	23.18	22.69
K (cmol _c dm ⁻³)	0.38	0.82	0.47	0.29
Ca (cmol _c dm ⁻³)	7.66	3.69	7.18	3.8
Mg (cmol _c dm ⁻³)	2.67	1.86	1.42	1.45
Al (cmol _c dm ⁻³)	0.04	0.12	0.04	1.58
H + Al (cmol _c dm ⁻³)	5.38	4.94	4.1	9.75
SB (cmol _c dm ⁻³)	10.71	6.36	9.07	5.55
CEC – pH 7,0 (cmol _c dm ⁻³)	16.09	11.3	13.17	15.3

SOM – Soil organic matter; P – Phosphorus; K – Potassium; Ca – Calcium; Mg – Magnesium; Al – Aluminum; H+Al – Potential acidity; SB – Sum of bases; CEC – Cation exchange capacity (pH 7.0).

Source: research data.

Experiments followed a randomized complete block design (RCBD) with five treatments and six replicates. Two treatments received 75% of the recommended phosphorus dose (P₂O₅) in the seeding row: 1) positive control, inoculated with the commercial product BiomaPhos[®] (P75-BiomaPhos); and 2) inoculated with the bacterial consortium *A. brasilense* and *P. fluorescens* (P75-Biofree). The third treatment received 100% of the recommended P₂O₅ dose without inoculation (P100-NI). The fourth treatment, negative control, received neither phosphate fertilization nor inoculation (P0-NI). Exclusively in the soybean experiment, a treatment with 75% of the P₂O₅ dose without inoculation (P75-NI) was included.

In soybean, except for P0-NI, all treatments received standard inoculation with *Bradyrhizobium japonicum* (SEMIA 5080) via seed treatment at doses of 100 mL for 50 kg of seeds (P75-BiomaPhos) and 300 mL for 50 kg of seeds (P75-Biofree). In corn, inoculation in the P75-BiomaPhos treatment was via seeds (100 mL per 60,000-seed sack), while in P75-Biofree, application occurred in the seeding furrow (300 mL ha⁻¹, flow rate of 160 L ha⁻¹).

Each experimental unit consisted of 10 rows of 6.0 m length, with 0.45 m row spacing, totaling 27 m². Seeding was performed under no-till system after desiccation with glyphosate (720 g ha⁻¹ a.i.) 15 days prior. Base fertilization included 90 kg ha⁻¹ of potassium (K₂O) and P₂O₅ according to treatments. Agrochemical applications were made for pest, disease, and weed management. In corn, nitrogen fertilization (200 kg ha⁻¹ N) was applied as top-dressing in a single dose between V4 and V6 stages (Table 1).

The product Biofree[®] (registration PR 001593-8.000001) contains 1×10^8 CFU mL⁻¹ of strains *A. brasilense* Ab-V6 (CNPSo 2084) and *P. fluorescens* CCTB (CNPSo 2719). The positive control, BiomaPhos[®] (registration PR 000497-9.000063), contains 4×10^9 CFU mL⁻¹ of strains *Bacillus subtilis* (CNPMS B2084; BRM034840) and *Bacillus megaterium* (CNPMS B119; BRM033112).

According to Normative Instructions SDA N^o. 13 and SDA N^o. 53, minimum agronomic parameters for registration of plant growth promoters were evaluated. In soybean, grain yield, 1,000-grain weight (MMG), plant height, shoot dry matter (MSPA), root dry matter (MSR), number of pods and grains per plant, grain yield (kg ha⁻¹), number and dry weight of nodules (MSN), and P concentration in grains and leaves were measured. In corn, yield, MMG, percentage of damaged grains, height of first ear insertion, plant height, number of rows and grains per row/ear, dry matter (t ha⁻¹), and P concentration in grains and plants were evaluated.

Shoot dry matter (MSPA) was determined by collecting five plants from the usable area per plot at the R1 stage, weighing fresh mass, and drying 150 g subsample in a forced-air oven (65 °C, 72 h) to estimate dry matter per plant. In corn, plant and ear insertion heights were measured directly on three random plants from the usable area. At harvest, 10 plants per usable area were sampled for pod/grain (soybean) or row/grain (corn) counted.

Yield was estimated by harvesting four central rows per plot (discarding 0.5 m from ends), threshing, and drying to 13% (soybean) and 14% (corn) moisture. MMG was calculated from 300-grain subsamples per plot. In corn, the percentage of damaged grains was assessed in a 250 g subsample. P concentrations in grains and shoots followed Embrapa (2009).

Data were compiled and subjected to analysis of variance (ANOVA), with means compared by Duncan's test ($p \leq 0.05$) using ASSISTAT software.

3 Results and Discussion

3.1 Productive response and yield components

Co-inoculation of *Azospirillum brasilense* and *Pseudomonas fluorescens* (P75-Biofree) promoted consistent increases in grain yield and its key components in both soybean and corn during the 2019/2020 season.

For soybean, the P75-Biofree treatment resulted in the highest average yield (4,250.25 kg ha⁻¹). This value did not differ statistically from those observed in the full phosphate fertilization treatment (P100-NI; 4,233.75 kg ha⁻¹) or the inoculated control P75-BiomaPhos (4,175.75 kg ha⁻¹). However, P75-Biofree was significantly superior to the absolute control without phosphorus (P0-NI; 3,809.75 kg ha⁻¹), representing a 10.4% increase (440.5 kg ha⁻¹) (Table 3). These results corroborate observations with the literature, which reports yield increases of 3% to 16% in soybean via co-

inoculation with PGPB (Barbosa *et al.*, 2021; Zeffa *et al.*, 2020). Such gains are supported by multiple synergistic mechanisms acting from the rhizosphere to primary plant metabolism.

Table 2 – Evaluation of yield components (number of pods per plant, number of grains per plant, and 1,000-grain weight) and grain yield of soybean in response to BiomaPhos® (P75-BiomaPhos) and Biofree® (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil

Variable	Location	CV%	P0-NI	P75-NI	P75-BiomaPhos	P75-Biofree	P100-NI
Yield (kg ha ⁻¹)	Candói - PR	4.52%	3,426 b	3,673 a	3,754 a	3,781 a	3,761 a
	Guarapuava - PR	6.05%	4,000 b	4,160 b	4,545 a	4,604 a	4,579 a
	Lapa - PR	6.46%	5,022 b	5,019 b	5,534 a	5,608 a	5,735 a
	Sertão - RS	2.65%	2,791 b	2,834 b	2,870 b	3,008 a	2,860 b
Number of pods per plant	Candói - PR	13.92%	51.2 ^{ns}	48.1	41.7	48.7	45.1
	Guarapuava - PR	11.16%	34.1 c	37.0 bc	41.4 ab	44.4 ab	40.1 ab
	Lapa - PR	15.46%	48.1 ^{ns}	51.7	50.3	50.2	49.1
	Sertão - RS	9.06%	33.9 ^{ns}	34.4	34.3	33.3	34.3
Number of grains per plant	Candói - PR	18.84%	117.7 ^{ns}	101.9	99.3	95.1	108.7
	Guarapuava - PR	17.65%	88.5 ^{ns}	93.9	97.3	97.8	91.3
	Lapa - PR	13.77%	131.6 ^{ns}	141.4	130.2	134.0	129.8
	Sertão - RS	9.20%	72.1 ^{ns}	85.0	85.0	85.5	78.8
MMG (g)	Candói - PR	2.73%	144 ^{ns}	153	155	155	156
	Guarapuava - PR	5.72%	163 ab	153 b	170 a	169 a	170 a
	Lapa - PR	2.61%	221 ^{ns}	218	220	224	221
	Sertão - RS	1.48%	144 b	146 b	152 a	150 a	149 a

MMG – 1,000-grain weight, expressed in grams. ^{ns} indicates no statistical difference. Lowercase letters in mean tables indicate significant differences (Duncan $p \leq 0.05$) among inoculation treatments within each location.

Source: research data.

Analysis of soybean yield components revealed that the number of pods per plant was significantly influenced by treatments only in Guarapuava, where P75-Biofree achieved the highest average (44.4 pods/plant). In other locations, this parameter showed no statistical differences (Table 3). This pattern suggests that yield gain was mediated mainly by better grain filling and pod retention, rather than an increase in total pod number (Cerezini *et al.*, 2016).

This effect can be attributed to additional nitrogen supply via associative BNF by *A. brasilense*, which can contribute 20 to 30 kg N ha⁻¹ to soybean (Hungria *et al.*, 2013). This nitrogen, available mainly as ammonium and amino acids, is preferentially directed to reproductive structure development (Cassán; Díaz-Zorita, 2020). Concurrently, cytokinin production, such as zeatin, by *A. brasilense* may break apical dominance, favoring lateral branching and formation of more reproductive nodes with pod retention potential (Fumaki *et al.*, 2018).

The 1,000-grain weight (MMG), a critical indicator of grain filling, responded positively to co-inoculation in soybean in Guarapuava-PR. At this site, treatments P75-Biofree (169 g), P75-BiomaPhos (170 g), and P100-NI (170 g) formed a statistically superior group compared to P75-NI (153 g), with gains of 10.5% to 11.1% (Table 3).

This result corroborates observations that bacterial regulators coordinate grain filling optimization (Marchão *et al.*, 2025). Specifically, indole-3-acetic acid (IAA) synthesized via the indole-3-pyruvate pathway by *A. brasilense* modulates expression of ARF (Auxin Response Factors) and AUX/IAA gene families in the plant. This modulation results in greater cell division and tissue elongation, including those involved in grain development (Fukami; Cerny; Burland, 2018). The contribution of *P. fluorescens* to IAA, though less intense and preferentially via the indole-3-acetamide pathway, adds to this hormonal pool in the rhizosphere, creating an environment of continuous growth stimulation (Oberhänsli; Défago; Haas, 1991).

In corn, co-inoculation (P75-Biofree) also demonstrated efficacy, with an average yield of 14,013 kg ha⁻¹. This value was statistically superior to the absolute control P0-NI (12,570 kg ha⁻¹), representing an 11.5% increase (1,443 kg ha⁻¹), and equivalent to the full phosphate fertilization treatment (P100-NI; 13,898 kg ha⁻¹) (Table 4).

Similar results were reported by Di Salvo *et al.* (2018) and Hungria *et al.* (2010), who observed average increases of 20% to 35% in corn yield associated with PGPB-containing inoculants under reduced phosphorus doses.

Table 4 – Evaluation of yield components (number of grains per ear, number of grains per row, and 1,000-grain weight) and grain yield of corn in response to BiomaPhos[®] (P75-BiomaPhos) and Biofree[®] (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil

Variable	Location	CV%	P0-NI	P75-BiomaPhos	P75-Biofree	P100-NI
Yield (kg ha ⁻¹)	Candói - PR	4.10%	14,062 b	15,413 a	15,784 a	15,413 a
	Guarapuava - PR	4.71%	14,329 b	15,521 a	15,687 a	15,506 a
	Lapa - PR	2.95%	16,544 b	18,058 a	18,061 a	18,012 a
	Sertão - RS	12.27%	5,346 b	6,503 a	6,520 a	6,660 a
Number of grains per ear (units)	Candói - PR	6.52%	488.30 ^{ns}	528.70	532.00	525.00
	Guarapuava - PR	6.18%	476.34 ^{ns}	500.74	499.92	485.53
	Lapa - PR	3.43%	535.31 c	544.85 bc	576.45 a	566.79 ab
	Sertão - RS	10.86%	337.92 ^{ns}	349.35	351.05	350.03
Number of grains per row (units)	Candói - PR	4.58%	31.40 ^{ns}	33.60	33.40	32.90
	Guarapuava - PR	4.82%	30.28 ^{ns}	31.13	31.13	30.80
	Lapa - PR	2.76%	33.17 c	33.85 bc	35.58 a	34.73 ab
	Sertão - RS	7.77%	22.33 ^{ns}	23.00	23.25	23.17
MMG (g)	Candói - PR	2.73%	423.19 ^{ns}	425.89	427.66	428.93
	Guarapuava - PR	2.03%	448.37 ^{ns}	453.40	462.62	458.69
	Lapa - PR	2.60%	454.70 ^{ns}	469.47	463.10	460.04
	Sertão - RS	3.57%	384.41 ^{ns}	374.31	389.08	376.22

MMG – 1,000-grain weight, expressed in grams. ^{ns} indicates no statistical difference. Lowercase letters in mean tables indicate significant differences (Duncan $p \leq 0.05$) among inoculation treatments within each location.

Source: research data.

The response of corn yield components to co-inoculation was significantly positive in Lapa-PR. At this location, P75-Biofree promoted significant increases in average number of grains per ear

(576.45) and per row (35.58) (Table 4). These results demonstrate the contribution of co-inoculation to corn reproductive development, acting decisively during critical stages of floral differentiation and grain formation. These stages (V5–VT) require high nitrogen and phosphorus supply to sustain floral primordia formation and embryo development (Lizaso *et al.*, 2018; Mi *et al.*, 2016). Thus, additional N supply via associative BNF by *A. brasilense* and increased P availability solubilized by *P. fluorescens* likely reduced ovule and flower abortion, resulting in a higher number of grains per ear (Hungria *et al.*, 2010; Richardson *et al.*, 2011).

However, the 1,000-grain weight (MMG) of corn showed no statistical difference among treatments and locations (Table 4). This result suggests that the yield increase was due mainly to formation of more grains rather than increased individual grain weight. This indicates that co-inoculated plants maintained an adequate source–sink balance, with sufficient photosynthetic capacity to meet the additional demand of grains without compromising their individual mass (Borrás; Gambín, 2010). Moreover, co-inoculation may have reduced ovule abortion chances during the grain “lag” growth phase (14–21 days post-pollination), a process highly sensitive to carbon and nutritional stress (Boyer; Westgate, 2004). The presence of PGPB may also modulate expression of genes associated with cell division and expansion, such as those in the CYC (cyclins) and CDK (cyclin-dependent kinases) families, and genes involved in cytokinin biosynthesis, including IPT (isopentenyl transferase) and LOG (Lonely Guy), which catalyze the final activation of these phytohormones. During grain filling, upregulation of these genes contributes to intensifying meristematic activity and assimilate transport, strengthening the plant’s capacity to allocate carbon and nutrients to reproductive sinks (Kudoyarova *et al.*, 2019; Glick, 2012).

3.2 Plant Architecture and Vegetative Development

The effect of co-inoculation extended to vegetative development of the crops, positively influencing dry biomass in soybean and corn.

For soybean, shoot dry matter (SDM) was significantly influenced by the P75-Biofree treatment (96 g) only in Guarapuava-PR (Table 5). At this site, co-inoculation surpassed the controls P75-NI (67 g) and P0-NI (61 g), with increases exceeding 40% (43.3% for P75-NI and 52.2% for P0-NI). This result aligns with observations by Marchão *et al.* (2025), who reported 10–18% increases in plant biomass via co-inoculation. This effect can be explained by coordinated hormonal action of the two bacteria and greater carbon assimilation and photosynthetic efficiency (Cerezini *et al.*, 2016). Plant physiology studies show that IAA produced by *A. brasilense* stimulates endogenous cytokinin biosynthesis in the plant, particularly zeatin and isopentenyladenine. These hormones act in maintaining photosynthetic activity and delaying leaf senescence (Cassán; Díaz-Zorita, 2016), an effect particularly relevant during grain filling, when maintenance of photosynthetically active leaves

is crucial for photoassimilate supply.

Table 5 – Evaluation of plant biomass (shoot dry matter, root dry matter, and nodule dry matter) and nodule number in soybean in response to BiomaPhos® (P75-BiomaPhos) and Biofree® (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil

Variable	Location	CV%	P0-NI	P75-NI	P75-BiomaPhos	P75-Biofree	P100-NI
SDM (g)	Candoi - PR	14.12%	138 ^{ns}	166	169	158	158
	Guarapuava - PR	19.67%	61 ^b	67 ^b	94 ^a	96 ^a	98 ^a
	Lapa - PR	13.06%	167 ^{ns}	175	181	187	193
	Sertão - RS	13.23%	98 ^{ns}	108	108	108	133
RDM (g)	Candoi - PR	25.39%	1.10 ^{ns}	1.44	1.26	1.37	1.40
	Guarapuava - PR	-	-	-	-	-	-
	Lapa - PR	16.86%	1.59 ^{ns}	1.63	1.76	1.63	1.60
	Sertão - RS	28.52%	1.62 ^{ns}	1.55	1.45	1.47	1.72
Number of nodules	Candoi - PR	23.24%	46.1 ^{ns}	53.2	60.3	56.5	56.6
	Guarapuava - PR	40.96%	39.0 ^{ns}	56.3	51.3	56.0	65.0
	Lapa - PR	24.71%	37.5 ^{ns}	56.4	53.8	55.8	58.8
	Sertão - RS	28.61%	28.5 ^{ns}	29.5	32.0	30.6	32.4
NDM (g)	Candoi - PR	54.50%	46.1 ^{ns}	53.2	60.3	56.5	56.6
	Guarapuava - PR	53.71%	151.0 ^{ns}	203.0	219.0	208.0	218.0
	Lapa - PR	21.89%	242.0 ^{ns}	310.0	343.0	323.0	343.0
	Sertão - RS	37.41%	148.0 ^{ns}	158.0	161.0	228.0	198.0

SDM – shoot dry matter, expressed in grams. RDM – root dry matter, expressed in grams. NDM – nodule dry matter, expressed in grams. ^{ns} indicates no statistical difference. Lowercase letters in mean tables indicate significant differences (Duncan $p \leq 0.05$) among inoculation treatments within each location.

Source: Research data.

Nodulation parameters, although showing no statistical difference, demonstrated a consistent numerical increase trend in the P75-Biofree treatment, with an average 38.9% increase in nodule dry matter (NDM) relative to P0-NI (Table 5).

This result reflects an important aspect of rhizosphere hormonal modulation. Studies with *P. fluorescens* mutants deficient in ACC deaminase production showed that this enzyme is fundamental for establishing tripartite symbiosis, reducing ethylene-mediated nodulation inhibition by up to 60% under stress conditions (Glick, 2012).

Concurrently, exogenous IAA produced by *A. brasilense* acts as a signal for expression of nodulation genes (ENOD40 and NIN), facilitating *Bradyrhizobium* infection (Fukami; Cerny; Burland, 2018).

The variability observed in root dry matter (RDM) parameters across locations, despite no statistical difference, may be attributed to interaction between PGPB and native microbial communities. Hungria *et al.* (2013) demonstrated that inoculated bacteria efficacy depends on their ability to establish in the rhizosphere by competing with resident microbiota.

In sites like Candoi-PR and Sertão-RS, where P75-Biofree showed higher MSR values than P75-BiomaPhos, more efficient integration likely occurred between inoculated bacteria and native beneficial microorganisms, resulting in a more functional rhizosphere.

For corn, co-inoculation promoted balanced and physiologically efficient vegetative growth.

This was reflected in increased dry matter production (MS/ha) without significant change in plant stature (Table 6). The P75-Biofree treatment showed 8% to 20% increases in MS/ha relative to P0-NI, reaching 18,433 kg ha⁻¹ in Candoi-PR and 24,472 kg ha⁻¹ in Lapa-PR. This increase suggests greater photoassimilate accumulation and enhanced energy metabolism, results consistent with additional nitrogen supply via biological fixation and improved efficiency in using phosphorus made available by the solubilizing rhizobacterium (Alori; Glick; Babalola, 2017; Fukami; Cerezini; Hungria, 2018).

Table 6 – Evaluation of corn vegetative development (height of first ear insertion, plant height, number of rows) and plant dry matter (MS/ha) in response to BiomaPhos[®] (P75-BiomaPhos) and Biofree[®] (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil.

Variable	Location	CV%	P0-NI	P75-BiomaPhos	P75-Biofree	P100-NI
MS/ha (kg ha ⁻¹)	Candoi - PR	9.68%	15,409 b	18,188 a	18,433 a	18,229 a
	Guarapuava - PR	8.50%	13,642 ^{ns}	14,293	15,054	14,829
	Lapa - PR	5.65%	22,274 b	24,159 a	24,472 a	24,095 a
	Sertão - RS	15.10%	9,059 ^{ns}	9,959	10,329	10,165
Ear insertion height (cm)	Candoi - PR	6.60%	119.00 ^{ns}	127.00	123.00	132.00
	Guarapuava - PR	8.37%	119.10 ^{ns}	122.34	124.46	123.33
	Lapa - PR	4.50%	147.04 ^{ns}	145.06	145.49	146.76
	Sertão - RS	9.97%	100.61 ^{ns}	97.09	98.92	94.83
Plant height (cm)	Candoi - PR	3.75%	233.00 ^{ns}	240.00	234.00	240.00
	Guarapuava - PR	4.00%	240.45 ^{ns}	240.32	237.77	240.45
	Lapa - PR	2.80%	279.00 ^{ns}	275.17	278.70	280.00
	Sertão - RS	8.53%	198.54 ^{ns}	192.34	202.07	190.08
Rows (units)	Candoi - PR	3.52%	15.5 ^{ns}	15.7	15.9	15.9
	Guarapuava - PR	2.86%	15.73 ^{ns}	16.08	16.03	15.77
	Lapa - PR	1.61%	16.13 ^{ns}	16.10	16.20	16.30
	Sertão - RS	5.12%	15.13 ^{ns}	15.20	15.08	15.03

MS – plant dry matter, expressed in kilograms per hectare.

^{ns} indicates no statistical difference. Lowercase letters in mean tables indicate significant differences (Duncan $p \leq 0.05$) among inoculation treatments within each location.

Source: research data.

Plant height and first ear insertion height in corn showed no statistical differences among treatments and locations (Table 6). These results indicate that biomass increase was associated with structural strengthening and leaf expansion, rather than excessive stem elongation. This growth pattern is agronomically desirable, as it reduces lodging risk and improves light interception (Hungria et al., 2010). Fukami; Cerezini; Hungria (2018) state that PGPB-inoculated plants show increased leaf area index (LAI) and better leaf angular distribution, optimizing photosynthetically active radiation use. Such responses suggest that the microbial consortium induced reorganization in photoassimilate allocation, favoring support structures and photosynthetic surfaces over vertical growth, resulting in a more efficient canopy.

3.3 Phosphorus concentration in leaf and grain tissues

Tissue phosphorus analysis revealed a fundamental aspect of inoculant mode of action. Co-inoculation with the bacterial consortium (P75-Biofree) did not significantly alter internal nutrient concentration in plant tissues, both foliar and grain, in soybean (Table 7) and corn (Table 8). This result is important, as it may indicate that the inoculants' mechanism primarily enhances phosphorus use efficiency. Co-inoculation allowed the plant to produce more dry biomass and grains for the same amount of absorbed P, promoting optimization in partitioning of absorbed P. The nutrient was preferentially directed to formation of new structural and reserve tissues, rather than simple accumulation in leaf tissues (Pereira *et al.*, 2020). According to Moretti *et al.* (2024), PGPB can increase P use efficiency by up to 25% via modulation of carbohydrate metabolism and assimilate partitioning—precisely the pattern observed here, consistent with enhanced root activity and internal P recycling as described in Moretti's study on soybean root dehydrogenase activity and nutrient uptake under PGPB influence.

Table 7 – Determination of foliar and grain P concentration in soybean in response to BiomaPhos[®] (P75-BiomaPhos) and Biofree[®] (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil

Variable	Location	CV%	P0-NI	P75-NI	P75-BiomaPhos	P75-Biofree	P100-NI
Foliar P concentration (g kg ⁻¹)	Candói - PR	8.73%	3.55 ^{ns}	3.52	3.58	3.54	3.51
	Guarapuava - PR	7.93%	3.20 ^{ns}	3.18	3.22	3.19	3.17
	Lapa - PR	9.81%	3.47 ^{ns}	3.83	3.47	3.47	3.55
	Sertão - RS	4.56%	5.87 ^{ns}	5.75	5.79	5.67	5.37
Grain P concentration (g kg ⁻¹)	Candói - PR	10.50%	2.52 ^{ns}	2.62	2.59	2.56	2.57
	Guarapuava - PR	7.41%	2.76 ^{ns}	2.74	2.75	2.72	2.77
	Lapa - PR	10.63%	2.48 ^{ns}	2.87	2.68	2.41	2.53
	Sertão - RS	8.44%	2.84 ^{ns}	2.69	2.82	2.61	2.88

Foliar P concentration – foliar phosphorus concentration, expressed in grams of phosphorus per kilogram of leaf. Grain phosphorus concentration, expressed in grams of phosphorus per kilogram of grain.

^{ns} indicates no statistical difference.

Source: Prepared by the authors (2026).

In soybean, the P75-Biofree treatment showed average foliar P (3.97 g kg⁻¹) and grain P (2.58 g kg⁻¹) slightly lower than P75-BiomaPhos (4.02 g kg⁻¹ and 2.71 g kg⁻¹, respectively) (Table 7). The latter, formulated with *Bacillus* spp., exhibited higher values, reflecting the direct phosphate solubilization capacity of this bacterial genus (Vey *et al.*, 2025). In contrast, the *P. fluorescens* mechanism predominantly involves solubilization of inorganic phosphates via localized rhizosphere acidification. This process may not necessarily reflect higher P concentration in tissues, but rather greater momentary and cyclic nutrient availability at the soil–root interface, immediately utilized by the plant to sustain growth (Vey *et al.*, 2025; Marchão *et al.*, 2025).

In corn, variation in grain P concentration was site-specific, with significant difference only in Guarapuava (PR) (Table 8). The BiomaPhos[®] treatment (1.42 g kg⁻¹) showed slightly higher values than P75-Biofree (1.18 g kg⁻¹) (Table 8). This response may relate to *Bacillus* strain specificity and

their modes of action, which may have made P available more synchronously with the critical grain-filling phase (Yadav; Tarafdar, 2012; Malboobi *et al.*, 2009).

Table 8 – Evaluation of foliar and grain phosphorus concentration in corn in response to BiomaPhos® (P75-BiomaPhos) and Biofree® (P75-Biofree) inoculants combined with phosphate fertilization, across four distinct edaphoclimatic sites in Southern Brazil

Variable	Location	CV%	P0-NI	P75-BiomaPhos	P75-Biofree	P100-NI
Foliar P concentration (g kg ⁻¹)	Candoi - PR	15.09%	3.19 ^{ns}	3.16	2.87	3.21
	Guarapuava - PR	10.15%	3.96 ^{ns}	4.08	4.02	4.23
	Lapa - PR	9.45%	4.34 ^{ns}	5.37	4.96	4.89
	Sertão - RS	15.19%	4.16 ^{ns}	4.49	4.49	4.07
Grain P concentration (g kg ⁻¹)	Candoi - PR	35.06%	0.55 ^{ns}	0.61	0.80	0.66
	Guarapuava - PR	17.66%	0.88 ^b	1.42 ^a	1.18 ^a	1.28 ^a
	Lapa - PR	50.98%	1.16 ^{ns}	1.13	0.97	1.02
	Sertão - RS	37.93%	2.24 ^{ns}	2.25	2.89	2.02

Foliar P concentration – foliar phosphorus concentration, expressed in grams of phosphorus per kilogram of leaf. Grain phosphorus concentration, expressed in grams of phosphorus per kilogram of grain. ^{ns} indicates no statistical difference. Lowercase letters in mean tables indicate significant differences (Duncan $p \leq 0.05$) among inoculation treatments within each location.

Source: Prepared by the authors (2026).

Overall, the performance of the *A. brasilense* and *P. fluorescens* consortium for both crops indicates more efficient redistribution of absorbed P. Co-inoculation prioritized essential physiological processes such as vegetative growth, floral differentiation, and grain establishment over P accumulation in tissues. This dynamic reflects a metabolically tuned plant capable of allocating nutrients according to physiological demand, maximizing productive efficiency (Santos; Nogueira; Hungria, 2019). Thus, the P75-Biofree treatment demonstrates that co-inoculation enabled significant phosphate fertilizer savings without yield loss, representing agronomic and environmental gain in tropical systems.

3.4 Synergistic Mechanisms and Metabolic Integration

The results obtained in this study demonstrate that co-inoculation of *A. brasilense* and *P. fluorescens* (P75-Biofree) promoted functional integration between rhizosphere and physiological processes. This integration resulted in greater nutritional efficiency and productive performance, even with a 25% reduction in phosphate fertilization. This effect is supported by the complementarity of each microorganism's modes of action, acting coordinately from nutrient uptake to shoot metabolic regulation (Fukami; Cerezini; Hungria, 2018; Hungria *et al.*, 2010; Vacheron *et al.*, 2022).

At the rhizosphere level, determining biochemical interactions occur, *A. brasilense*, one of the most efficient bacterial IAA producers, synthesizes this hormone predominantly via the indole-3-pyruvate (IPyA) pathway through the enzyme indole-3-pyruvate decarboxylase (IPDC). Bacterial IAA acts synergistically with plant endogenous IAA, stimulating cell elongation and formation of

longer and more numerous root hairs, as well as promoting lateral root initiation (Fukami; Cerezini; Hungria, 2018; Olanrewaju; Glick; Babalola, 2019; Vacheron et al., 2022). The effect is not only morphological but functional: roots with greater specific surface area and soil volume explored exhibit higher nutrient foraging and absorption capacity (Hungria et al., 2010).

Simultaneously, *P. fluorescens* act by solubilizing phosphates and mobilizing iron. The bacterium excretes organic acids such as gluconate and 2-ketogluconate, which lower rhizosphere pH and dissolve Fe and Al phosphates common in acidic soils (Kalayu, 2019; Vyas; Gulati, 2009). Additionally, production of high affinity siderophores such as pyoverdine and pyochelin chelates Fe³⁺, increasing its availability to the plant while releasing P previously complexed to these ions (Vansuyt et al., 2007).

These mechanisms positively feedback. The increase in root density and extension stimulated by *A. brasilense* expands the action zone of *P. fluorescens*. In turn, *P. fluorescens* intensifies P availability, sustaining new root growth and closing a cycle of increasing physiological efficiency. This process explains yield maintenance with 75% of the P dose, as the consortium makes the plant more efficient in exploring soil P and residual fertilizer (Richardson et al., 2011; Pereira et al., 2020).

At the metabolic and physiological level, interaction favors integration of nitrogen and phosphorus pathways. N supply by associative BNF of *A. brasilense* stimulates synthesis of structural and enzymatic proteins involved in phosphorus transport and metabolism (Hu et al., 2019). Conversely, P released by *P. fluorescens* provides the energy (ATP) needed to sustain BNF metabolism and biomass formation. This N–P balance results in a plant with more stable and efficient metabolism, reflected in increased dry matter, grain number, and yield maintenance (Canarini et al., 2019).

At the hormonal signaling level, co-inoculation establishes an optimal balance between auxin, cytokinin, and ethylene favoring vegetative and reproductive growth (Shimoia et al., 2024; Spaepen; Vanderleyden; Remans, 2007). Studies with *A. brasilense* IAA-deficient mutants (*ipdC*⁻) demonstrated that auxin signaling is essential for growth-promoting effects (Dobbelaere et al., 1999; Fukami, Cerny; Burland, 2018). Additionally, modulation of ethylene levels by *P. fluorescens* ACC deaminase prevents growth inhibition under phosphate restriction (Husen et al., 2011; Mahadik; Kumudini., 2020).

Co-inoculation also appears to modulate gene expression involved in cell division, cytokinin biosynthesis and partitioning (Glick, 2012; Kudoyarova et al., 2019). In soybean, for example, co-inoculation positively regulates genes involved in sucrose transport (SWEET11) and starch biosynthesis (GBSSI), allowing better photoassimilate partitioning (Marchão et al., 2025; Shimoia et al., 2024; Xu et al., 2022). Complementary proteomic studies identified increased abundance of key enzymes in the TCA cycle and amino acid biosynthesis pathway in co-inoculated plants, suggesting

metabolic reprogramming that sustains higher yield and biomass (Bais *et al.*, 2023; Shimoia *et al.*, 2024).

The interaction among these different mechanisms creates a physiological “priming” effect, where the plant becomes more efficient in resource use even under suboptimal conditions (Kumar; Verma, 2018). This improved physiological state is reflected not only in yield, but also in crop resilience, as demonstrated by result consistency across different edaphoclimatic environments (Fukami; Cerny; Burland, 2018). These findings support the interpretation that the bacterial consortium acts through multiple integrated mechanisms resulting in greater overall resource use efficiency. validating the hypothesis that a 25% reduction in phosphate fertilizer dose is possible without compromising productivity.

4 Conclusions

Co-inoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* represents a promising biotechnological strategy for the sustainable production of soybean and corn in Brazil. The microbial consortium allowed a 25% reduction in phosphorus fertilization without compromising crop yield, resulting in increases of 10.4% (440 kg ha⁻¹) and 11.5% (1,443 kg ha⁻¹) for soybean and corn, respectively, compared with the absolute control in the 2019/2020 growing season under different edaphoclimatic conditions. These results indicate that this technology can enhance the physiological efficiency of plants through microbial synergy, representing an economically advantageous alternative that combines higher yields with reduced costs and environmental impacts, contributing to more sustainable and profitable agricultural systems.

Acknowledgements

The authors thank Biotrop Soluções Biológicas for providing the microbial inoculants (Biofree[®] and BiomaPhos[®]) and technical support during field trials.

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