

Article

Soil Management Modulates Maize Responses to Phosphate-Solubilizing Bacteria

João Vitor Silvério Alves de Avelar ¹, Ubiraci Gomes de Paula Lana ², Raquel Gomes de Oliveira ¹ , Mariana Lourenço Campolino ², Isabela Figueiredo de Oliveira ¹ , Thiago Teixeira Santos ³ , Eliane Aparecida Gomes ², Christiane Abreu de Oliveira-Paiva ² , Rafael Felipe Ratke ⁴  and Sylvia Moraes de Sousa ^{1,2,*} 

¹ Campus Dom Bosco, Federal University of São João del-Rei (UFSJ), São João del-Rei 36301-158, MG, Brazil; joaovt.silverio@gmail.com (J.V.S.A.d.A.); raqgoliveira@gmail.com (R.G.d.O.); isabela.engguim@outlook.com (I.F.d.O.)

² Embrapa Maize and Sorghum, Sete Lagoas 35701-970, MG, Brazil; ubiraci.lana@embrapa.br (U.G.d.P.L.); mlcampolino@gmail.com (M.L.C.); eliane.a.gomes@embrapa.br (E.A.G.); christiane.paiva@embrapa.br (C.A.d.O.-P.)

³ Embrapa Digital Agriculture, Campinas 13083-886, SP, Brazil; thiago.santos@embrapa.br

⁴ Campus de Chapadão do Sul, Federal University of Mato Grosso do Sul (UFMS), Chapadão do Sul 79560-000, MS, Brazil; rafael.ratke@ufms.br

* Correspondence: sylvia.sousa@embrapa.br

Abstract

Phosphorus (P) limitation constrains crop production in highly weathered tropical soils, and microbial inoculants may enhance P acquisition, although their effectiveness varies with edaphic conditions. We hypothesized that the effects of a phosphate-solubilizing bacterial inoculant on plant performance and belowground processes would depend on soil P status and no-tillage history. A two-year field experiment on an Oxisol in the Brazilian Cerrado (Chapadão do Sul, MS, Brazil) compared newly established, low-P (NTS-NC) and consolidated, high-P (NTS-C) no-tillage systems. Treatments combined 0, 50, or 100% of recommended P fertilization with or without BiomaPhos[®], composed of *Priestia megaterium* CNPMS B119 and *Bacillus subtilis* CNPMS B2084. Grain yield, resin-extractable soil phosphorus (P-resin), root architecture, bacterial and arbuscular mycorrhizal fungal (AMF) communities, AMF colonization, and phosphatase activities were evaluated. Grain yield in NTS-C exceeded NTS-NC by 15.7% and 18.8% in 2021/2022 and 2022/2023, respectively. Inoculation × P interactions for yield and P-resin occurred only in NTS-NC in the second season. Inoculation altered root architecture, whereas P availability reorganized bacterial association networks. AMF responses were limited and season-specific, while inoculation reduced AMF colonization and phosphatase activity primarily in NTS-C. These findings confirm that inoculant performance is context-dependent and should be integrated with site-specific P fertilization strategies.

Keywords: arbuscular mycorrhizal fungi; bacterial association networks; bioinoculant; Cerrado; phosphatase activity; rhizosphere; root system architecture; *Zea mays* L.



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1. Introduction

Phosphorus (P) availability remains a major constraint on crop production in highly weathered tropical soils, where strong phosphate adsorption onto iron and aluminum oxides limits plant P acquisition [1,2]. Although P fertilization is essential for maintaining

crop productivity in these soils, improving the use of both fertilizer-derived and residual soil P is important for increasing P-use efficiency and reducing dependence on continued fertilizer inputs [3]. Phosphate-solubilizing microorganisms (PSMs) have therefore received increasing attention as a complementary strategy for improving plant P acquisition [4,5].

PSMs can mobilize poorly available P through organic acid production, phosphatases, phytases, and other mechanisms [4,5]. Some plant growth-promoting bacteria can also modify root development and interact with resident rhizosphere microorganisms, indicating that plant responses to inoculation may involve processes beyond direct P solubilization [6]. In Brazil, this approach led to the development of BiomaPhos[®], containing *Priestia megaterium* CNPMS B119 and *Bacillus subtilis* CNPMS B2084. Field trials have reported an average maize grain yield increase of approximately 8.9% following inoculation [7,8]. However, responses are not consistent across environments, and recent field evidence indicates substantial variation in plant, root, and rhizosphere responses to these strains between growing seasons [9].

This variability raises an important question for the field application of microbial inoculants: which soil and management conditions favor their agronomic and biological effects? Introduced microorganisms encounter resident microbial communities and soil environments shaped by nutrient availability and management history [10]. No-tillage systems offer a useful field context for addressing this question because recently established and long-term consolidated systems can differ in soil organic matter, nutrient cycling, physical properties, and microbial organization [11–13]. Yet, whether such contrasting conditions modify maize responses to PSB inoculation, and the belowground processes associated with those responses, remains insufficiently understood.

Assessing this question requires measurements beyond grain yield or extractable soil P. Root system architecture, rhizosphere bacterial communities, arbuscular mycorrhizal fungi (AMF), and phosphatase activity represent complementary components through which plants and microorganisms can influence P acquisition [1,4,6]. Simultaneously evaluating these components can therefore help determine whether field responses to inoculation are driven primarily by changes in soil P availability or by broader modifications at the soil–root–microbiome interface.

In this study, we evaluated maize responses to P fertilization and BiomaPhos[®] inoculation over two growing seasons in recently established, low-P and consolidated, high-P no-tillage systems. We integrated grain yield and resin-extractable soil phosphorus (P-resin) with root system architecture, rhizosphere bacterial community structure, AMF colonization and community structure, and rhizosphere phosphatase activity. We hypothesized that the magnitude and biological expression of maize responses to PSB inoculation would differ between no-tillage systems with contrasting management histories and P availability, and that these differences would be reflected in root and rhizosphere processes rather than solely in extractable soil P. This framework allowed us to test how contrasting field contexts influence the agronomic and belowground responses associated with PSB inoculation.

2. Materials and Methods

2.1. Study Site and Experimental Design

Field experiments were conducted during the 2021/2022 and 2022/2023 growing seasons at the Experimental Farm of the Federal University of Mato Grosso do Sul, Chapadão do Sul, Mato Grosso do Sul State, Brazil (18°46′17.9″ S, 52°37′25.0″ W; 810 m a.s.l.), located in the Cerrado region. According to the Köppen climate classification, the regional climate is Aw (tropical savanna with a dry winter), with a mean annual temperature of approximately 24–25 °C and annual precipitation ranging from approximately 1600 to 1850 mm.

Rainfall is strongly seasonal, with the rainy season extending predominantly from October to April and the dry season from May to September [14].

The soil in the experimental area was classified as a Typic Dystrophic Red Latosol (Latossolo Vermelho distrófico típico), corresponding to an Oxisol, according to the Brazilian Soil Classification System [15]. Two no-tillage systems (NTS) contrasting in management history and soil P availability were evaluated: (i) a long-term consolidated no-tillage system (NTS-C), under no-tillage management for more than 10 years and characterized by high soil P availability (High-P); (ii) a recently established no-tillage system (NTS-NC), converted to no-tillage one year before the beginning of the experiment and characterized by low soil P availability (Low-P). Soil P status was determined based on the initial soil fertility analysis (Table 1).

Table 1. Analysis of soil samples collected from the experimental area of a Red Oxisol at a depth of 0–20 cm under non-consolidated (NTS-NC) and consolidated (NTS-C) no-tillage systems in Mato Grosso do Sul State, Brazil.

Depth (cm)		Cmolc dm ^{−3}								H ₂ O	
NTS-NC	0–20	Ca + Mg	K	Ca	Mg	H + Al	Al	CEC	V%	SB	pH
		4.9	0.5	3.9	1	4.2	0	9.6	56.25	5.4	4.5
		mg dm ^{−3}				g kg ^{−1}					
		K	P	Cu	Fe	Mn	Zn	OM	Clay	Silt	Sand
		196	5	1.3	148	16.7	3.5	30	480	25	495
Depth (cm)		Cmolc dm ^{−3}								H ₂ O	
NTS-C	0–20	Ca + Mg	K	Ca	Mg	H + Al	Al	CEC	V%	SB	pH
		3.8	0.4	3.39	0.43	4.224	0	8.4	49.9578	4.2	5.98
		mg dm ^{−3}				g kg ^{−1}					
		K	P	Cu	Fe	Mn	Zn	OM	Clay	Silt	Sand
		156.4	10.9	0.85	69.99	13.45	134.5	26.87	480	25	495

Ca + Mg: calcium plus magnesium; K: potassium; Ca: calcium; Mg: magnesium; H + Al: potential acidity (hydrogen plus aluminum); Al: aluminum; CEC: cation exchange capacity; V: base saturation; SB: sum of exchangeable bases (Ca²⁺, Mg²⁺, and K⁺); pH (H₂O): soil pH measured in water; P (resin): resin-extractable phosphorus; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; OM: organic matter; Sand: sand content; Silt: silt content; Clay: clay content. Units are expressed as cmolc dm^{−3} for exchangeable cations and acidity, mmolc dm^{−3} for CEC, mg dm^{−3} for extractable nutrients, dag kg^{−1} for OM, and g kg^{−1} for soil particle-size fractions.

At each site, the experiment followed a randomized complete block design with three replicates and 20 m² plots. Treatments were arranged in a 3 × 2 factorial design with three P fertilization rates and two inoculation treatments (non-inoculated and inoculated with PSB).

2.2. Phosphorus Fertilization and Microbial Inoculation

The commercial phosphate-solubilizing microbial inoculant BiomaPhos® (Bioma, Cruz Alta, RS, Brazil), containing *Priestia megaterium* CNPMS B119 and *Bacillus subtilis* CNPMS B2084, at a concentration of approximately 4 × 10⁹ viable cells mL^{−1}, was applied to maize seeds at a rate of 100 mL per 60,000 seeds (equivalent to 100 mL ha^{−1}), corresponding to an estimated dose of approximately 6.7 × 10⁶ viable bacterial cells per seed. Seed inoculation was performed immediately before sowing following the procedure described by de Oliveira-Paiva et al. [8].

Phosphorus fertilization rates were established according to the initial soil P status and the fertilizer recommendation for maize under the fertility conditions of each no-tillage

system. Treatments were designated P0, P50, and P100, representing 0, 50, and 100% of the recommended P_2O_5 rate for each system, respectively. In the consolidated no-tillage system (NTS-C; high-P), P0, P50, and P100 corresponded to 0, 25, and 50 kg P_2O_5 ha⁻¹, respectively, whereas in the newly established no-tillage system (NTS-NC; low-P), they corresponded to 0, 50, and 100 kg P_2O_5 ha⁻¹, respectively. Triple superphosphate (46% P_2O_5) was used as the P source.

2.3. Crop Management and Agronomic Evaluations

Maize hybrid P3551PWU (Pioneer®, Corteva Agriscience do Brasil Ltd., Barueri, SP, Brazil) was grown during the 2021/2022 growing season, whereas hybrid FS615PWU (Forseed®, LongPing High-Tech, Cravinhos, SP, Brazil) was grown during the 2022/2023 growing season. The hybrid change between growing seasons was necessary because seeds of P3551PWU were not available for the second experimental season. Within each growing season, the same hybrid was used across all experimental treatments.

Weed control was initiated 10 days before maize sowing with glyphosate (720 g a.i. ha⁻¹) plus haloxyfop-P-methyl (63 g a.i. ha⁻¹). In both growing seasons, maize was mechanically sown at approximately 3 cm depth, with 0.45 m row spacing and a density of 3.5 seeds m⁻¹. At 20 days after emergence (DAE), post-emergence weed control was performed with atrazine (1.5 L a.i. ha⁻¹) plus tembotrione (101 g a.i. ha⁻¹). Mineral oil at 0.5% (v/v) was added to the spray solution.

Nitrogen and potassium fertilization were applied at the V6 growth stage at rates of 120 kg N ha⁻¹ as urea (45% N) and 120 kg K₂O ha⁻¹ as potassium chloride (60% K₂O), respectively. Both fertilizers were manually broadcast onto the soil surface of each experimental plot. At the onset of flowering, pyraclostrobin + epoxiconazole was applied at rates of 99.7 + 37.5 g a.i. ha⁻¹, respectively, for rust control. The insecticides methomyl and acetamiprid + bifenthrin were applied at rates of 129 and 30 + 30 g a.i. ha⁻¹, respectively, for the control of caterpillars and stink bugs.

At physiological maturity, maize ears were manually harvested from a 2-m² area within the central rows of each plot and mechanically shelled using a stationary sheller. Grain mass was determined for the harvested area, adjusted to 13% moisture content, and converted to grain yield expressed as kg ha⁻¹.

2.4. Rhizosphere Soil Sampling and Phosphorus Determination

Rhizosphere soil samples were collected from the root system at maize flowering in each growing season. We collected four subsamples from each plot and combined them into one composite sample. Resin-extractable soil phosphorus (P-resin) was determined by the Brazilian Laboratory of Environmental and Agricultural Analysis (LABRAS, Monte Carmelo, Brazil) using its standardized ion-exchange resin procedure. The method is based on the removal of phosphate from the soil solution by ion-exchange resin, promoting the desorption of labile P from the soil solid phase. The P retained by the resin is subsequently extracted and quantified as an estimate of soil P availability.

2.5. Root System Architecture Analysis

Root system architecture (RSA) was evaluated at flowering using the Shovelomics approach [16]. Three maize plants were randomly selected at different positions along the two outer rows of each plot. The central rows were preserved for grain yield determination to avoid disturbance of plant stand and potential interference with yield estimates. The three sampled plants were analyzed individually as subsamples, and trait values were averaged to obtain a single value for each experimental plot. Thus, the experimental unit for RSA analysis was the plot, with three biological replicates corresponding to the three experimental blocks.

For each selected plant, the root crown was carefully excavated around the plant base to a depth of 20 cm. Root crowns were washed to remove adhering soil and photographed according to the procedures described in reference [17]. Images were analyzed using the Digital Imaging of Root Traits (DIRT) platform, version 5.2 [18], with the modifications described in reference [19]. The root architectural traits obtained from image analysis are described in Table 2.

Table 2. Root system architecture (RSA) traits [17].

RSA Trait	Abbreviation
Projected root area	AREA
Average root density	AVG_DENSITY
Median root system width	WIDTH_MED
Cumulative root width at 10% depth	D10
Skeleton root depth	SKL_DEPTH
Top root angle	ANG_TOP
Average lateral root diameter	LT_AVG_DIA

2.6. Rhizosphere Microbial Community Analysis

Rhizosphere soil collected from three plants within each plot was pooled to obtain one composite sample per experimental unit. Total soil DNA was extracted from 0.45 g of rhizosphere soil using the DNeasy® PowerSoil Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions.

Bacterial communities were characterized by amplification of the 16S *rRNA* gene, whereas arbuscular mycorrhizal fungal (AMF) communities were assessed through nested PCR amplification of the 28S *rRNA* gene using previously validated primer sets [20–22].

Microbial community structure was evaluated using terminal restriction fragment length polymorphism (T-RFLP). Amplified fragments were digested with *AluI*, *HaeIII*, and *HhaI* restriction enzymes (Invitrogen, Carlsbad, CA, USA) and analyzed using an ABI 3500XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Consensus terminal restriction fragments (T-RFs) were generated using T-REX software v1.0 [23], and only fragments representing $\geq 1\%$ of the total relative abundance were retained for subsequent analyses. T-RFLP was used as a community fingerprinting approach to evaluate relative shifts in rhizosphere bacterial and AMF community structure across contrasting no-tillage systems, P fertilization rates, inoculation treatments, and growing seasons.

Putative taxonomic assignment of bacterial T-RFs was performed using the Microbial Community Analysis (MiCA III) platform (v1.0) and cross-checked against the NCBI Taxonomy database following the procedure described in reference [19]. Shannon–Wiener (H') [24], Simpson (D) [25], and Chao [26] were calculated using R software based on the bacterial phyla and families identified in each treatment.

2.7. Arbuscular Mycorrhizal Fungal Colonization

Fine fresh roots (~1 g) were collected from maize plants, immersed in 70% (*v/v*) ethanol, and stored at 4 °C for 3 days. Roots were then washed with deionized water and cleared overnight in 10% (*w/v*) potassium hydroxide (KOH) at room temperature (RT). After clearing, the roots were thoroughly rinsed with deionized water and acidified in 0.3 M hydrochloric acid (HCl) for 30 min at RT. Root samples were subsequently stained for 30 min at RT with 0.01% (*w/v*) trypan blue prepared in a solution containing 2.5% (*v/v*) acetic acid and 50% (*v/v*) glycerol. Stained roots were transferred to conical tubes containing acidified glycerol (1:1 glycerol and 0.3 M HCl) and stored until microscopic evaluation.

Arbuscular mycorrhizal fungal (AMF) colonization was quantified using the grid-line intersect method described in reference [27], with modifications according to reference [28]. Root samples were examined using an Axio Zoom V16 stereomicroscope (Zeiss, North York, ON, Canada) at 20× magnification. A total of 100 root intersections per sample were evaluated for the presence or absence of AMF structures, including arbuscules, vesicles, and hyphae. Total AMF colonization was expressed as the percentage of examined intersections containing mycorrhizal fungal structures.

2.8. Soil Phosphatase Activity

Acid and alkaline phosphatase activities were determined in rhizosphere soil according to reference [29]. Briefly, soil samples were sieved through a 2 mm mesh and homogenized. Enzyme activity was assayed using *p*-nitrophenyl phosphate (*p*NPP) as substrate in modified universal buffer adjusted to pH 6.5 and 11.0 for acid and alkaline phosphatase, respectively. Samples were incubated at 37 °C for 1 h, and phosphatase activity was determined colorimetrically by quantifying the *p*-nitrophenol (*p*NP) released from the substrate. Absorbance was measured at 400 nm, and enzyme activity was calculated from a *p*NP standard curve and expressed as $\mu\text{g } p\text{-nitrophenol h}^{-1} \text{ g}^{-1} \text{ soil}$.

2.9. Statistical Analysis

Data were assessed for normality and homogeneity of variances before statistical analysis. Agronomic variables, soil P availability, root system architecture traits, phosphatase activities, bacterial diversity indices, relative abundances of bacterial taxa, and arbuscular mycorrhizal fungal (AMF) root colonization were subjected to analysis of variance (ANOVA). Within each no-tillage system and growing season, P fertilization, microbial inoculation, and their interaction were considered fixed effects. When significant effects were detected ($p \leq 0.05$), treatment means were compared using Tukey's test. AMF root colonization percentages were arcsine square-root-transformed prior to ANOVA to meet model assumptions. Community structure of bacteria and AMF was evaluated using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities, and differences among treatments were assessed using analysis of similarities (ANOSIM) in PAST v3.25 [30].

Pearson's correlation coefficients were calculated to assess relationships among agronomic, soil, root architectural, and rhizosphere biological variables, and correlation patterns were examined separately for each no-tillage system and growing season. Principal component analysis (PCA) was subsequently performed using selected agronomic, soil, root, and rhizosphere biological variables to examine multivariate relationships among treatments and identify the variables contributing most strongly to treatment differentiation. Bacterial co-occurrence networks were constructed separately for each no-tillage system, growing season, and P fertilization level. Microbial associations were inferred using Sparse Correlations for Compositional data (SparCC), and associations with an absolute correlation coefficient $|r| \geq 0.30$ were retained for network construction. Nodes represented bacterial taxa, and edges represented significant associations, with positive and negative correlations distinguished by the sign of the correlation coefficient. Network topology was characterized using network density, clustering coefficient, modularity, and natural connectivity.

Statistical analyses and data visualization were performed in R version 4.4.0 (R Core Team, 2024) using agricolae (de Mendiburu [31]), ExpDes.pt (Ferreira et al. [32]), corrplot (Wei and Simko [33]), rstatix (Kassambara [34]), ggplot2 (Wickham [35]), factoextra (Kassambara and Mundt [36]), tidyverse (Wickham et al. [37]), and Hmisc (Harrell et al. [38]). Co-occurrence network analyses and visualization were performed using igraph (Csárdi et al. [39]) and ggraph (Pedersen [40]).

3. Results

3.1. No-Tillage Context Shapes Maize Productivity and the Agronomic Response to PSB Inoculation

Maize grain yield differed markedly between the two no-tillage systems, whereas responses to PSB inoculation and P fertilization varied with growing season and management context (Figure 1; Tables S1 and S2). During 2021/2022, neither PSB inoculation nor P fertilization significantly affected grain yield or resin-extractable soil phosphorus (P-resin) in the newly established no-tillage system (NTS-NC). In the consolidated no-tillage system (NTS-C), P fertilization showed only a tendency to affect grain yield, whereas neither inoculation nor the inoculation $\times$ P fertilization interaction significantly affected grain yield or P-resin (Tables S1 and S2).

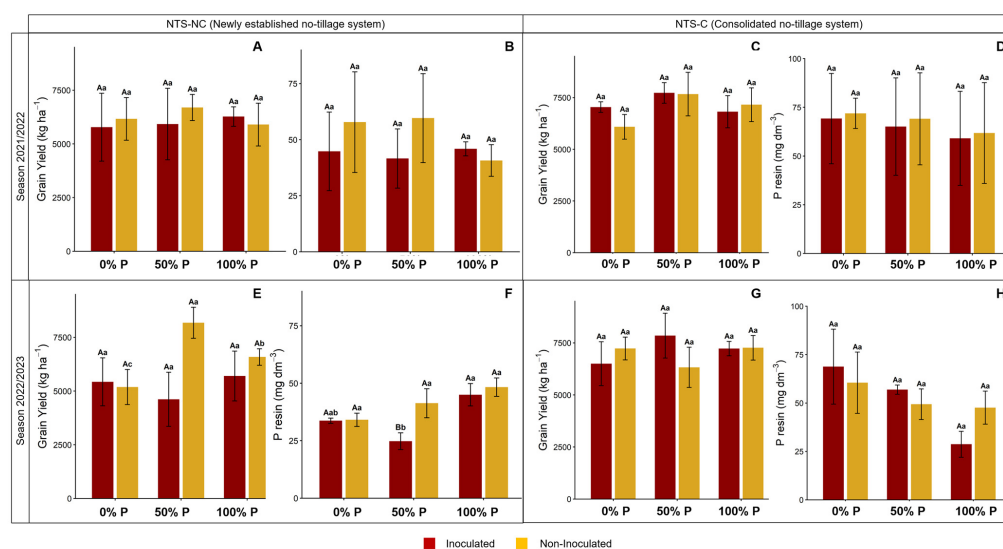


Figure 1. Effects of PSB inoculation and phosphorus fertilization on maize grain yield and soil P availability under contrasting no-tillage systems. Maize grain yield (A,C,E,G) and resin-extractable soil phosphorus (P-resin; (B,D,F,H)) as affected by PSB inoculation and phosphorus (P) fertilization during the 2021/2022 and 2022/2023 growing seasons. Panels (A,B,E,F) represent the newly established no-tillage system (NTS-NC), whereas panels (C,D,G,H) represent the consolidated no-tillage system (NTS-C). Phosphorus treatments P0, P50, and P100 correspond to 0, 50, and 100% of the recommended P fertilization rate for each no-tillage system, respectively. Inoculated treatments received BiomaPhos®, containing the phosphate-solubilizing bacteria *Priestia megaterium* CNPMS B119 and *Bacillus subtilis* CNPMS B2084, whereas non-inoculated treatments received no inoculant. Uppercase letters compare inoculated and non-inoculated treatments within each P fertilization rate, whereas lowercase letters compare P fertilization rates within each inoculation treatment. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

A different response emerged during 2022/2023. In NTS-NC, significant inoculation $\times$ P fertilization interactions were detected for both grain yield and P-resin, indicating that the response to PSB depended on P supply (Figure 1; Tables S1 and S2). In contrast, no corresponding interaction was detected in NTS-C, where inoculation had little effect on either grain yield or P-resin across P fertilization rates.

Despite these treatment-dependent responses, grain yield differed consistently between the two no-tillage systems. Grain yield was 15.7% higher in NTS-C than in NTS-NC in 2021/2022 and 18.8% higher in 2022/2023 (Figure 1). More importantly, the response to P fertilization and PSB inoculation differed between the two no-tillage systems, with significant inoculation $\times$ P interactions detected in NTS-NC during the second growing season but not in NTS-C.

3.2. Maize Root System Architecture Responds to PSB Inoculation in a P-, Management-, and Season-Dependent Manner

Root system architecture (RSA) was more responsive to PSB inoculation and P fertilization than grain yield, although the magnitude and direction of these responses depended on growing season and no-tillage system (Figure 2; Tables S3–S6). During 2021/2022, treatment effects on RSA were limited. In NTS-NC, P fertilization significantly affected only top root angle (ANG_TOP), whereas average root density (AVG_DENSITY) was the only trait significantly affected by P supply in NTS-C. No consistent effect of inoculation on RSA was detected during this growing season (Tables S3 and S4).

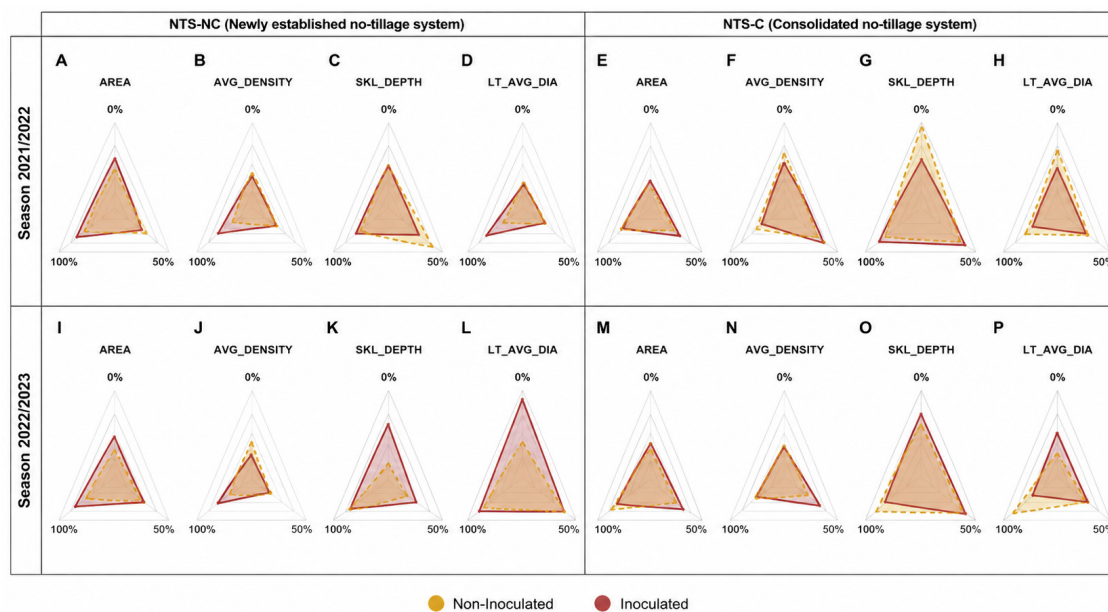


Figure 2. Maize root system architecture responses to PSB inoculation and phosphorus fertilization depend on no-tillage context and growing season. Selected maize root system architecture traits in PSB-inoculated and non-inoculated treatments under P0, P50, and P100, corresponding to 0, 50, and 100% of the recommended phosphorus (P) fertilization rate for each no-tillage system, respectively. Panels (A–H) represent the newly established no-tillage system (NTS-NC) and consolidated no-tillage system (NTS-C), respectively, during the 2021/2022 growing season, whereas panels (I–P) represent NTS-NC and NTS-C, respectively, during the 2022/2023 growing season. Root system architecture traits are projected root area (AREA; (A,E,I,M)), average root density (AVG_DENSITY; (B,F,J,N)), root skeleton depth (SKL_DEPTH; (C,G,K,O)), and average lateral root diameter (LT_AVG_DIA; (D,H,L,P)). Each radar plot shows three vertices: P0, P50, and P100. Colors distinguish PSB-inoculated and non-inoculated treatments, as indicated in the figure.

During the 2022/2023 growing season, significant root architectural responses to the experimental treatments were detected, particularly in NTS-NC. PSB inoculation significantly affected projected root area (AREA), median root system width (WIDTH_MED), root skeleton depth (SKL_DEPTH), and average lateral root diameter (LT_AVG_DIA), whereas P fertilization affected most of the root architectural traits evaluated (Tables S5 and S6). Significant inoculation $\times$ P fertilization interactions were detected for AVG_DENSITY, SKL_DEPTH, and LT_AVG_DIA, indicating that the effect of inoculation on these root traits varied with P fertilization level.

In NTS-NC, inoculated plants exhibited greater root depth under low and intermediate P supply and increased lateral root diameter at specific P fertilization levels (Figure 2; Tables S5 and S6). Root density also showed an inoculation-dependent response to P supply, with interaction patterns detected in both no-tillage systems. Treatment effects on the remaining architectural traits were smaller or less consistent. Collectively, these results

indicate that maize RSA was more sensitive to PSB inoculation than grain yield, particularly during the second growing season and in the newly established, low-P no-tillage system.

3.3. Phosphorus Availability and PSB Effects on Bacterial Community Structure Depend on Management Context and Growing Season

Rhizosphere bacterial community structure responded to both P fertilization and PSB inoculation, although the magnitude and consistency of these responses depended on no-tillage context and growing season (Figures 3 and S1).

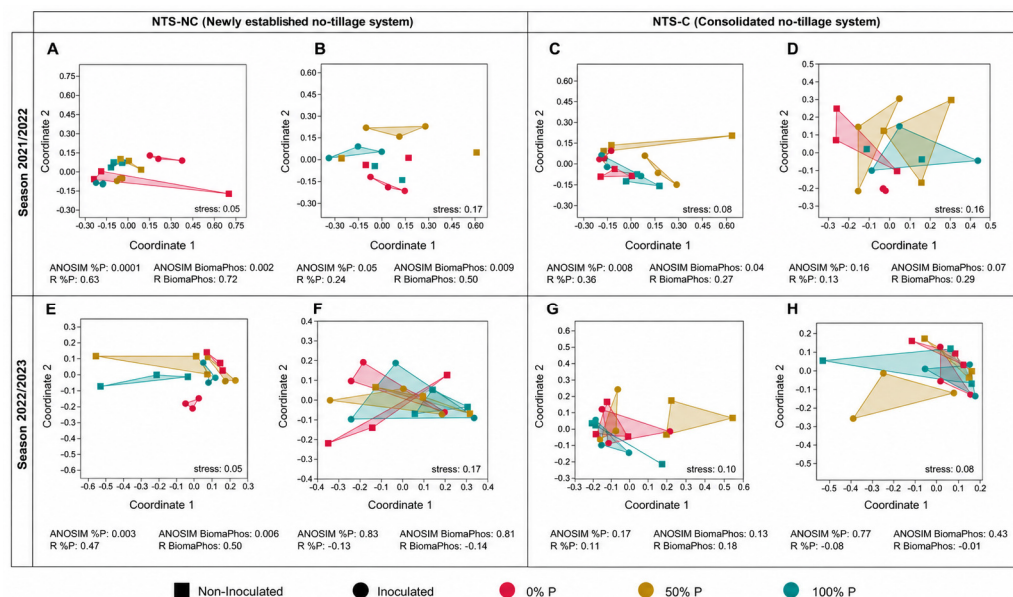


Figure 3. Rhizosphere bacterial and arbuscular mycorrhizal fungal community responses to PSB inoculation and phosphorus fertilization under contrasting no-tillage systems. Non-metric multi-dimensional scaling (NMDS) ordination of bacterial (A,C,E,G) and arbuscular mycorrhizal fungal (AMF; (B,D,F,H)) community profiles based on Bray–Curtis dissimilarities during the 2021/2022 (A–D) and 2022/2023 (E–H) growing seasons. Panels (A,B,E,F) represent the newly established no-tillage system (NTS-NC), whereas panels (C,D,G,H) represent the consolidated no-tillage system (NTS-C). Squares and circles represent non-inoculated and PSB-inoculated treatments, respectively, whereas colors represent P0, P50, and P100, corresponding to 0, 50, and 100% of the recommended phosphorus (P) fertilization rate for each no-tillage system. Polygons delimit treatment groups according to P fertilization level. Differences in community structure associated with P fertilization and PSB inoculation were assessed by analysis of similarities (ANOSIM); the corresponding *p*- and *R*-values are shown below each panel. NMDS stress values are shown within each ordination. Bacterial communities were characterized by amplification of the *16S rRNA* gene, whereas AMF communities were assessed by nested PCR amplification of the *28S rRNA* gene using previously validated primer sets.

During 2021/2022, treatment effects on bacterial community structure were most pronounced in NTS-NC. NMDS ordination showed separation among P fertilization treatments, and ANOSIM confirmed significant effects of both P fertilization ($p = 0.0001$, $R = 0.63$) and PSB inoculation ($p = 0.002$, $R = 0.72$) (Figure 3A). In NTS-C, both P fertilization ($p = 0.008$, $R = 0.36$) and inoculation ($p = 0.04$, $R = 0.27$) also significantly affected bacterial community structure, although these effects were weaker than in NTS-NC (Figure 3C). Separate NMDS visualizations by inoculation and P fertilization further illustrated these treatment-dependent patterns (Figure S1A–D).

Actinomycetota, Bacillota, Cyanobacteriota, and Pseudomonadota predominated in the bacterial profiles (Tables S7 and S8). At the family level, bacterial community composition showed marked temporal shifts in both no-tillage systems (Figure 4). In

and the balance between positive and negative associations varied substantially among treatments (Figure 5M,N).

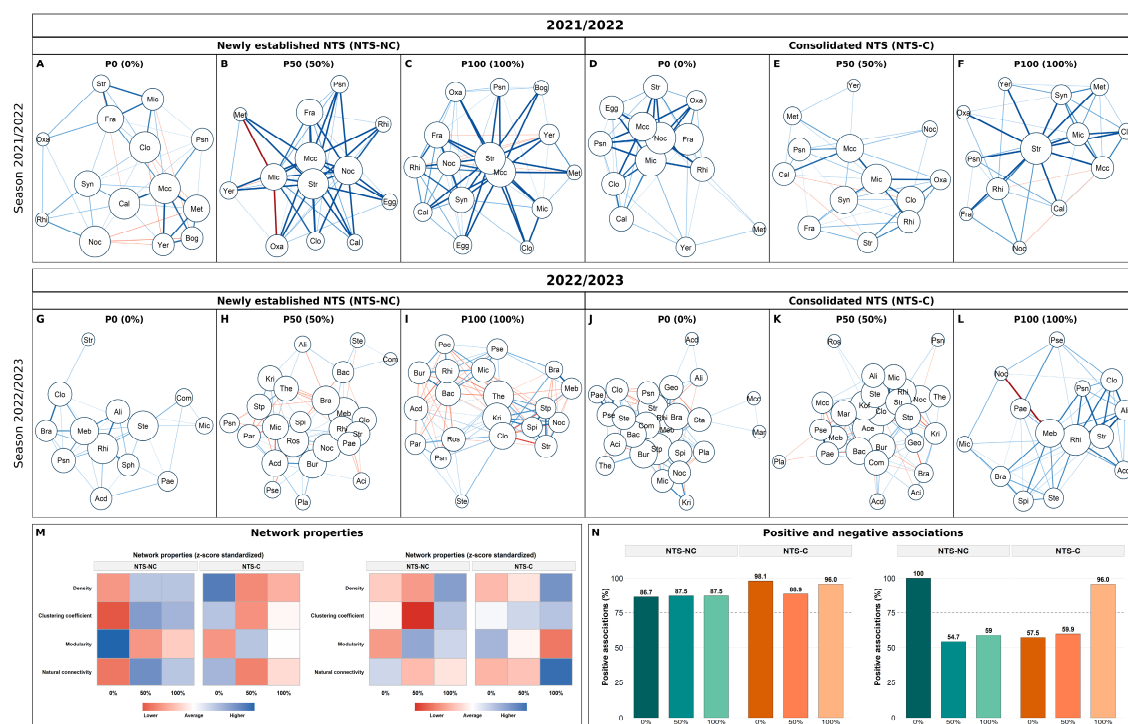


Figure 5. Phosphorus availability reorganizes bacterial co-occurrence networks in the maize rhizosphere under contrasting no-tillage systems and growing seasons. Co-occurrence networks of bacterial families in the maize rhizosphere under P0, P50, and P100, corresponding to 0, 50, and 100% of the recommended phosphorus (P) fertilization rate for each no-tillage system, during the 2021/2022 (A–F) and 2022/2023 (G–L) growing seasons. Panels (A–C,G–I) represent the newly established no-tillage system (NTS-NC), whereas panels (D–F,J–L) represent the consolidated no-tillage system (NTS-C); within each system, panels correspond sequentially to P0, P50, and P100. Networks were constructed using the combined bacterial community dataset from PSB-inoculated and non-inoculated plots within each P fertilization × no-tillage system combination. Nodes represent bacterial families, with node size proportional to node degree. Edges represent significant associations between bacterial families; blue and red edges indicate positive and negative associations, respectively, and edge thickness is proportional to association strength. (M) Heatmap of network topological properties, including density, clustering coefficient, modularity, and natural connectivity, standardized within each growing season. (N) Proportion of positive associations within each network according to P fertilization level and no-tillage system. Bacterial family abbreviations for 2021/2022: Bog, Bogoriellaceae; Cal, Caldicellulosiruptoraceae; Clo, Clostridiaceae; Egg, Eggerthellaceae; Fra, Frankiaceae; Met, Methylophilaceae; Mic, Microbacteriaceae; Mcc, Micrococcaceae; Noc, Nocardiaceae; Oxa, Oxalobacteraceae; Psn, Pseudonocardiaceae; Rhi, Rhizobiaceae; Str, Streptomycetaceae; Syn, Synechococcaceae; Yer, Yersiniaceae; and Oth, other families. Bacterial family abbreviations for 2022/2023: Ace, Acetobacteraceae; Aci, Acidimicrobiaceae; Acd, Acidithiobacillaceae; Alc, Alcaligenaceae; Ali, Alicyclobacillaceae; Bac, Bacillaceae; Bog, Bogoriellaceae; Bra, Bradyrhizobiaceae; Bru, Brucellaceae; Bur, Burkholderiaceae; Chl, Chlorobiaceae; Clo, Clostridiaceae; Com, Comamonadaceae; Cys, Cystobacteriaceae; Des, Desulfatibacillaceae; Dsf, Desulfatobacteriaceae; Geo, Geobacteraceae; Kof, Kofleriaceae; Kri, Kribbellaceae; Mar, Marinobacteraceae; Meb, Methylobacteriaceae; Mic, Microbacteriaceae; Mcc, Micrococcaceae; Mor, Moraxellaceae; Nit, Nitrobacteraceae; Noc, Nocardiaceae; Ncd, Nocardiodaceae; Oxa, Oxalobacteraceae; Pae, Paenibacillaceae; Par, Paracoccaceae; Phy, Phyllobacteriaceae; Pla, Planctomycetaceae; Pse, Pseudomonadaceae; Psn, Pseudonocardiaceae; Rhi, Rhizobiaceae; Rho, Rhodobacteraceae; Ros, Roseobacteraceae; Sph, Sphaerotilaceae; Spi, Sphingomonadaceae; Sta, Staphylococcaceae; Ste, Streptococcaceae; Str, Streptomycetaceae; Stp, Streptosporangiaceae; The, Thermomonosporaceae; and Oth, other families.

During 2021/2022, network organization responded differently to P availability in the two no-tillage systems. In NTS-NC, the P0 network showed lower density, clustering, and natural connectivity, and higher modularity than networks receiving P fertilization. P50 produced the strongest shift toward a more densely connected and less modular network, whereas P100 maintained relatively high network integration (Figure 5A–C,M). In contrast, the NTS-C network was most densely connected and least modular under P0. At P50, connectivity decreased, and modularity increased, with P100 showing an intermediate pattern (Figure 5D–F,M). Thus, P fertilization promoted greater network integration in NTS-NC, whereas the opposite pattern was observed in NTS-C, where the most integrated network occurred without P addition.

Positive associations strongly predominated across all bacterial networks during 2021/2022, accounting for 86.7–98.1% of the detected associations (Figure 5N). The most highly connected bacterial families also shifted with P supply and no-tillage context. Micrococcaceae showed high connectivity in NTS-NC at P0 and P50, whereas Streptomycetaceae became prominent at P100. In NTS-C, the highest-degree families shifted from Nocardiaceae at P0 to Microbacteriaceae at P50 and Streptomycetaceae at P100 (Figure 5A–F).

Network organization changed substantially during 2022/2023, with stronger variation in both network size and the balance between positive and negative associations. In NTS-NC, topological properties responded nonlinearly to P supply: P50 produced the most compartmentalized network, whereas P100 favored a more densely connected structure (Figure 5G–I,M). In NTS-C, increasing P availability was associated with progressively lower modularity and greater network integration. Although the P100 network contained fewer bacterial families than P0 and P50, it was more densely connected and less compartmentalized (Figure 5J–L,M).

The balance between positive and negative associations also differed markedly between growing seasons (Figure 5N). In NTS-NC, all detected associations were positive under P0 during 2022/2023, whereas positive associations decreased to approximately 55% and 59% under P50 and P100, respectively. In NTS-C, positive associations accounted for approximately 58% and 60% at P0 and P50, respectively, but increased sharply to 96% at P100. Thus, P availability affected not only network topology but also the balance between positive and negative bacterial associations, and the direction of these responses differed between no-tillage systems.

Highly connected bacterial families also differed among treatments during 2022/2023. In NTS-NC, Rhizobiaceae showed the highest connectivity at P0, Methylobacteriaceae and Rhizobiaceae at P50, and Kribbellaceae at P100. In NTS-C, Methylobacteriaceae and Rhizobiaceae were prominent at P0, Rhizobiaceae and Streptomycetaceae at P50, and Rhizobiaceae at P100 (Figure 5G–L).

Collectively, these results demonstrate that P availability substantially reorganized bacterial association patterns even when changes in overall community composition were comparatively small. Both the direction and magnitude of these responses depended on no-tillage context and growing season, indicating that the effects of P availability on rhizosphere bacterial network organization were strongly conditioned by soil management history and environmental context.

3.5. PSB Inoculation Differentially Affects AMF Communities, Root Colonization, and Phosphatase Activity Across No-Tillage Systems

Arbuscular mycorrhizal fungal community structure was generally less responsive to P fertilization and PSB inoculation than bacterial community structure (Figure 3). During 2021/2022, PSB inoculation significantly affected AMF community structure in NTS-NC ($p = 0.009$, $R = 0.50$), whereas the effect of P fertilization was marginal ($p = 0.05$, $R = 0.24$) (Figure 3B). In NTS-C, neither inoculation nor P fertilization significantly affected AMF

community structure (Figure 3D). During 2022/2023, neither factor significantly affected AMF community structure in either NTS-NC or NTS-C (Figure 3F,H).

Despite these limited changes in overall AMF community structure, AMF root colonization showed a contrasting response to inoculation depending on no-tillage context (Figure 6A). PSB-inoculated plants showed lower AMF root colonization than non-inoculated plants in NTS-C during both 2021/2022 and 2022/2023, whereas no significant inoculation effect was detected in NTS-NC in either growing season.

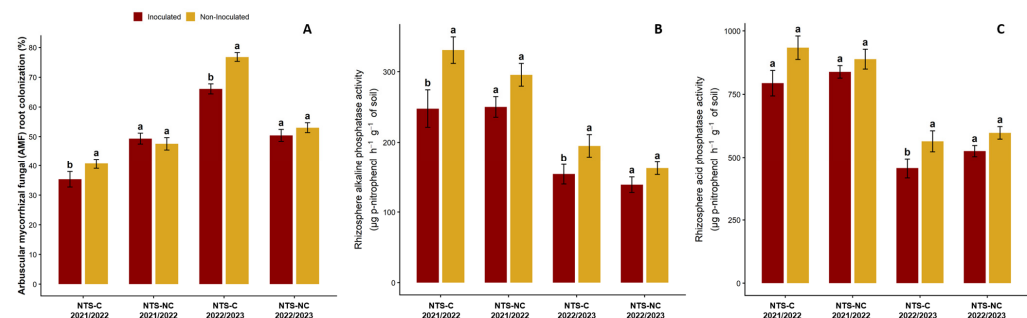


Figure 6. PSB inoculation alters arbuscular mycorrhizal colonization and rhizosphere phosphatase activity in a management- and season-dependent manner. Panels show (A) arbuscular mycorrhizal fungal (AMF) root colonization (%), (B) rhizosphere alkaline phosphatase activity, and (C) rhizosphere acid phosphatase activity in maize with (inoculated) and without (non-inoculated) phosphate-solubilizing bacteria (PSB) inoculation under newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 and 2022/2023 growing seasons. Phosphatase activities are expressed as $\mu\text{g p-nitrophenol (pNP) g}^{-1} \text{ soil h}^{-1}$. Different letters indicate significant differences between inoculated and non-inoculated treatments within each no-tillage system and growing season according to Tukey's test ($p \leq 0.05$).

Rhizosphere phosphatase activity showed a similarly management-dependent response (Figure 6B,C). No significant effects of PSB inoculation were detected for either alkaline or acid phosphatase activity in NTS-NC during either growing season. In NTS-C, however, inoculation significantly reduced alkaline phosphatase activity during both 2021/2022 and 2022/2023 (Figure 6B), whereas acid phosphatase activity was significantly reduced only during 2022/2023 (Figure 6C).

Overall, PSB inoculation affected different components of the AMF–phosphatase response depending on no-tillage context. Changes in AMF community structure were detected primarily in NTS-NC during 2021/2022, whereas reductions in AMF root colonization and phosphatase activity were concentrated in NTS-C. These contrasting responses further show that PSB inoculation effects on rhizosphere biological processes were strongly conditioned by management context and growing season.

3.6. Multivariate Integration Reveals Context-Dependent Relationships Among Root Architecture and Rhizosphere Biological Processes

Integration of plant, soil, and microbial variables revealed contrasting associations among PSB inoculation, root system architecture, and rhizosphere biological processes (Figure 7). Principal component analysis explained 67.7–70.5% of the total variation in the datasets, depending on growing season and no-tillage system. Across both growing seasons, inoculated treatments tended to align with root architectural traits, particularly projected root area (AREA), average root density (AVG_DENSITY), root skeleton depth (SKL_DEPTH), average lateral root diameter (LT_AVG_DIA), and root system width at 10% of the accumulated depth (D10). In contrast, phosphatase activities, P-resin, bacterial diversity, and AMF colonization were more frequently oriented toward non-inoculated treatments (Figure 7).

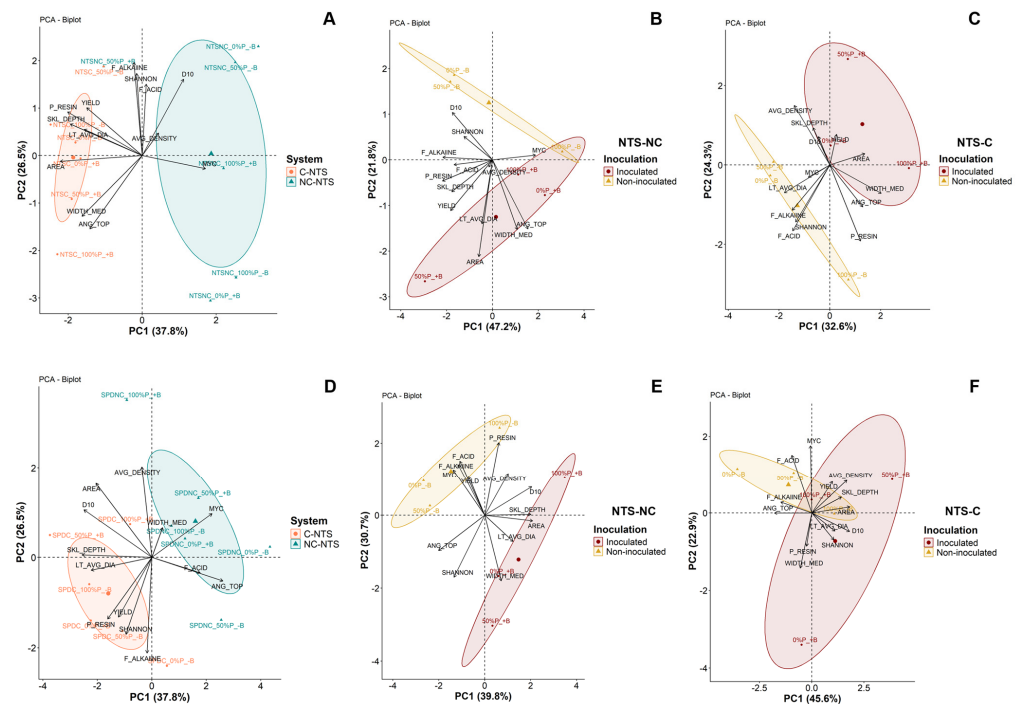


Figure 7. Integrated relationships among maize root architecture, agronomic performance, soil phosphorus availability, and rhizosphere biological properties. Principal component analysis (PCA) integrating plant, soil, and rhizosphere biological variables during the 2021/2022 (A–C) and 2022/2023 (D–F) growing seasons. Panels (A,D) show the overall ordination according to no-tillage system, comparing the newly established no-tillage system (NTS-NC) and the consolidated no-tillage system (NTS-C). Panels (B,E) show treatments within NTS-NC according to PSB inoculation, whereas panels (C,F) show the corresponding treatments within NTS-C. Treatments within each no-tillage system represent combinations of PSB inoculation (inoculated or non-inoculated) and P0, P50, and P100, corresponding to 0, 50, and 100% of the recommended phosphorus (P) fertilization rate for each system. Variables included projected root area (AREA), average root density (AVG_DENSITY), median root system width (WIDTH_MED), root system width at 10% of the accumulated depth (D10), root skeleton depth (SKL_DEPTH), top root angle (ANG_TOP), average lateral root diameter (LT_AVG_DIA), grain yield (YIELD), resin-extractable soil phosphorus (P_RESIN), arbuscular mycorrhizal fungal root colonization (MYC), Shannon–Wiener bacterial diversity index (SHANNON), acid phosphatase activity (F_ACID), and alkaline phosphatase activity (F_ALKALINE). Arrows represent variable loadings, with their direction and length indicating the direction and relative contribution of each variable to the ordination. Ellipses illustrate the distribution of treatments within the groups indicated in each panel. The percentages shown on the PC1 and PC2 axes indicate the proportion of total variance explained by each principal component.

Pearson correlation analyses further revealed season- and management-dependent relationships among plant, soil, and rhizosphere variables (Figure S2). During 2021/2022, grain yield was positively correlated with root skeleton depth in NTS-NC, whereas P-resin was positively associated with alkaline phosphatase activity (Figure S2A). In NTS-C, AMF colonization was positively associated with phosphatase activities, and bacterial Shannon diversity was positively related to both acid and alkaline phosphatase activities (Figure S2B).

During 2022/2023, the correlation structure shifted substantially. In NTS-NC, grain yield showed positive associations with P-resin and alkaline phosphatase activity, while several root architectural traits were more strongly intercorrelated (Figure S2C). In NTS-C, grain yield was positively associated with root architectural traits, particularly projected root area and average root density, whereas phosphatase activities showed contrasting relationships with root architecture and bacterial diversity (Figure S2D).

Together, the PCA and correlation analyses indicate that relationships among maize performance, root architecture, soil P availability, and rhizosphere biological processes varied markedly between no-tillage systems and growing seasons. PSB inoculation was more frequently associated with shifts in root architectural traits than with consistent increases in P-resin, grain yield, or bacterial diversity, whereas rhizosphere biological properties showed stronger dependence on soil management context and growing season (Figures 7 and S2).

4. Discussion

The agronomic response to phosphate-solubilizing bacteria (PSB) was not uniform across the contrasting no-tillage systems. Grain yield was consistently higher in the consolidated no-tillage system (NTS-C) than in the newly established system (NTS-NC), but this difference was expected given their contrasting soil-management histories and initial fertility conditions and, by itself, does not represent the main finding of the study. More importantly, the response of grain yield to PSB inoculation depended on P fertilization and field context. Significant inoculation $\times$ P fertilization interactions for grain yield and resin-extractable soil P (P-resin) emerged in NTS-NC during the second growing season, whereas comparable responses were not detected in NTS-C. Thus, our hypothesis that maize responsiveness to PSB would differ between contrasting no-tillage contexts was supported, although the agronomic benefit of inoculation was restricted to specific combinations of soil context, P fertilization, and growing season rather than representing a consistent main effect across field conditions.

The contrasting yield responses should be interpreted within the distinct soil environments represented by the two no-tillage systems. Long-term conservation management can improve soil functioning through continuous residue inputs, greater organic matter accumulation, improved aggregation and water retention, and enhanced nutrient cycling [11,12]. It can also alter P dynamics by promoting the accumulation and stratification of labile and organic P pools in surface soil layers [41], thereby increasing the contribution of residual or legacy P to crop nutrition. These characteristics, together with the higher initial P availability in NTS-C, likely contributed to its consistently greater productivity and may also have reduced the marginal benefit of introducing a P-solubilizing inoculant. In contrast, the lower-P NTS-NC provided a more restrictive nutritional background in which microbial effects on P acquisition could become more relevant. This interpretation is consistent with the inoculation $\times$ P interactions detected in this system and may help explain why microbial inoculants often produce variable agronomic responses across environments with contrasting baseline fertility [42,43].

Importantly, management history and initial soil P status were intrinsically associated under our experimental conditions. NTS-C represented both a longer-established no-tillage system and a higher-P soil, whereas NTS-NC combined a shorter management history with lower initial P availability. Their individual contributions therefore cannot be experimentally separated. The observed differences should consequently be interpreted as responses to contrasting soil-management and fertility contexts rather than as independent effects of no-tillage duration or soil P status. This distinction is particularly relevant when considering PSB inoculation because the effectiveness of an introduced microorganism depends not only on its functional capacity but also on the nutritional and ecological environment into which it is introduced [10,42,43]. Previous field experiments with inoculants containing the same PSB species, *Priestia megaterium* and *Bacillus subtilis*, have similarly demonstrated substantial variation in plant, soil, and microbial responses between growing seasons [9], supporting the view that inoculant performance is strongly context-dependent.

Root system architecture provides one possible biological pathway associated with this context dependence. Root architectural responses to inoculation were particularly evident in NTS-NC during the second experiment, when PSB affected traits related to root area, width, depth, and lateral root diameter, and several traits showed inoculation $\times$ P fertilization interactions. These responses are relevant because P has low mobility in soil, particularly in highly weathered tropical soils where sorption strongly restricts its availability to plants [1,2]. Root traits that modify the spatial distribution and volume of soil explored can therefore influence access to heterogeneous P pools [44,45]. In addition to their capacity to mobilize P, *Bacillus* and *Priestia* strains may produce indole-3-acetic acid and other metabolites capable of modifying root elongation, branching, and lateral root development [6,46,47]. Although these mechanisms were not directly quantified here, they provide a plausible biological basis for the association between PSB inoculation and changes in maize root architecture.

Notably, root architectural responses to inoculation were clearer than consistent changes in P-resin or grain yield. The integrated multivariate analyses similarly tended to associate inoculated treatments more closely with root architectural traits than with P-resin or productivity. This distinction suggests that the biological activity of a phosphate-solubilizing inoculant should not be evaluated exclusively by increases in extractable soil P. Changes in root exploration may contribute to plant P acquisition without necessarily producing a detectable increase in the bulk soil P pool measured by resin extraction. Likewise, modification of root phenotype does not necessarily translate into a yield gain when P is not the principal factor limiting crop production or when other soil and environmental constraints prevail. This interpretation is consistent with previous field observations in which PSB inoculation modified maize root traits and other P-acquisition-related responses without producing a consistent overall increase in grain yield [9]. Therefore, the present results indicate a partial decoupling between biological responses to inoculation and their final agronomic expression.

The rhizosphere bacterial community provides an additional ecological dimension to this interpretation. PSB inoculation did not produce broad and persistent shifts in overall bacterial community structure across both experiments, indicating that biological responses to the inoculant did not require major restructuring of the resident microbiome detectable by T-RFLP. Introduced microorganisms may influence nutrient mobilization or plant development without becoming dominant components of the resident community, and the relatively coarse taxonomic resolution of T-RFLP should also be considered when interpreting these patterns. In contrast, P availability was strongly associated with changes in bacterial community organization, particularly in the co-occurrence networks. Network density, modularity, connectivity, and the balance between positive and negative associations varied among P treatments, but the direction of these changes differed between NTS-NC and NTS-C. Thus, P supply did not impose a uniform effect on the rhizosphere microbiome; instead, its influence depended on the pre-existing soil-management and fertility context. This interpretation is consistent with evidence that no-tillage management can modify soil bacterial and fungal community structure and diversity [48].

These network responses are particularly informative because they reveal changes in community organization that were not always accompanied by comparable changes in bacterial α -diversity or overall community composition. During the second experiment, for example, treatment effects on these broader community metrics were relatively weak, whereas network topology varied substantially among P treatments and no-tillage systems. P availability may therefore reorganize patterns of bacterial association without necessarily causing large shifts in overall diversity or community fingerprints. Nevertheless, these networks represent statistical co-occurrence patterns and should not be interpreted as

evidence of direct microbial cooperation or competition. Positive and negative associations may arise from shared or contrasting environmental preferences, resource availability, indirect interactions, or potential metabolic dependencies. Thus, changes in network topology should be interpreted as changes in potential ecological association structure rather than as direct evidence of microbial interactions.

The responses of arbuscular mycorrhizal fungi and rhizosphere phosphatase activities further demonstrate that the different components of the P-acquisition system did not respond synchronously to PSB inoculation. AMF community structure was comparatively stable, with treatment effects restricted mainly to NTS-NC during the first experiment. In contrast, AMF root colonization was reduced by PSB inoculation in NTS-C during both experiments, while no comparable inoculation effect occurred in NTS-NC. Rhizosphere phosphatase activity showed a similar management-dependent pattern: inoculation reduced alkaline phosphatase activity in NTS-C in both experiments and acid phosphatase activity in the second experiment, whereas no significant inoculation effects were detected for these enzymes in NTS-NC. These responses indicate that introducing PSB does not necessarily stimulate all biological pathways associated with P acquisition simultaneously.

The higher initial P availability and longer management history of NTS-C may have altered the relative importance and energetic cost of different P-acquisition strategies. Long-term soil management can modify the distribution and forms of organic P [41], while plant investment in AMF symbiosis can decline with increasing P availability [49]. Extracellular phosphatase activity is also closely regulated by soil P conditions and microbial community composition [50]. The simultaneous reductions in AMF colonization and phosphatase activity following inoculation in NTS-C may therefore reflect changes in the relative contribution of different P-acquisition processes. However, these responses should not be interpreted as evidence that increased root development replaced or compensated for mycorrhizal or phosphatase-mediated P acquisition. The present experiment demonstrates co-occurring responses but does not establish causal relationships among root architecture, AMF colonization, phosphatase activity, P availability, and grain yield. Rather, the collective evidence indicates that PSB inoculation operates within an existing network of plant and microbial P-acquisition strategies whose relative responses depend on the soil environment.

An important limitation when interpreting differences between the two experiments is the use of different maize hybrids. P3551PWU was used in the first growing season, whereas FS615PWU was used in the second because seeds of the first hybrid were unavailable for the subsequent experiment. Maize genotypes can differ in root system architecture, nutrient-acquisition strategies, rhizosphere interactions, and responsiveness to microbial inoculation. Consequently, the stronger root responses and other biological differences observed in the second experiment cannot be attributed exclusively to interannual environmental variation or treatment effects. Because hybrid identity and growing season were confounded, their individual contributions cannot be separated, and differences between experiments may reflect genotype, environment, genotype $\times$ environment interactions, or their interactions with P fertilization and inoculation. This limitation is particularly relevant to root traits, for which genotype-dependent variation can be substantial [44,45]. Nevertheless, within each growing season, the same hybrid was used across all P fertilization and inoculation treatments. Therefore, treatment effects and inoculation $\times$ P interactions detected within a given experiment remain interpretable independently of hybrid identity, whereas direct comparisons of response magnitude between growing seasons should be made cautiously.

Taken together, the integrated analyses do not support a single sequential mechanism linking PSB inoculation to increased P availability and ultimately to higher grain yield. Instead, different components of the plant–soil–microbiome system responded at different organizational levels. PSB inoculation was most clearly associated with changes in root

architecture, whereas P availability exerted a stronger influence on the organization of resident bacterial communities. AMF colonization and phosphatase activities revealed an additional level of context dependence, particularly in the consolidated, higher-P system. The multivariate analyses reinforce these associations but should not be interpreted as demonstrating causal pathways. This distinction is important because measurable biological responses to inoculation may occur even when they do not translate directly into increased extractable soil P or crop productivity.

Overall, the absence of a uniform yield response to PSB across treatments is informative for defining the conditions under which microbial inoculation may provide an agronomic benefit. Our hypothesis was supported in that maize responses to PSB differed between contrasting soil-management and P-fertility contexts, but the mechanisms underlying this dependency could not be attributed to a single P-acquisition pathway. Under the conditions evaluated here, significant agronomic responses to inoculation emerged only under specific combinations of P fertilization, the newly established lower-P no-tillage context, and growing season, whereas root architectural responses were more broadly detectable. These findings emphasize that PSB inoculation should be considered a context-dependent component of integrated P management rather than a uniformly effective substitute for P fertilization. Identifying the soil fertility and management conditions under which biological responses are most likely to translate into yield benefits will be essential for improving the targeting and predictability of microbial inoculants in tropical cropping systems.

5. Conclusions

The field performance of phosphate-solubilizing bacteria cannot be predicted from their functional potential alone, as their agronomic effects depend on the soil-management and fertility context in which they are applied. In this study, PSB inoculation did not produce a uniform agronomic benefit, indicating that biological responses to inoculation do not necessarily translate into increased grain yield. Instead, the results support a context-dependent response in which baseline P availability, management history, and growing-season conditions influence the opportunity for microbial inoculation to contribute to crop performance.

The integration of agronomic, root, soil, and microbial responses further indicates that PSB activity should not be interpreted solely through changes in extractable soil P. Root architectural responses and shifts in rhizosphere P-acquisition processes occurred even in the absence of consistent yield responses, suggesting that multiple biological pathways contribute to the plant response but that their agronomic expression depends on the prevailing soil environment. Because management history was confounded with initial P status, and growing season with maize hybrid, the relative contribution of these factors could not be separated and should be addressed in future multi-environment experiments using a common genotype.

These findings have direct implications for the agronomic use of microbial inoculants: PSB should be integrated into site-specific P-management strategies rather than considered a stand-alone replacement for P fertilization. Identifying soil fertility thresholds and management conditions under which microbial responses translate into yield benefits will be critical for improving the predictability and effective deployment of PSB-based technologies in tropical cropping systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16181861/s1>, Figure S1: Effects of PSB inoculation and phosphorus fertilization on rhizosphere bacterial community structure under contrasting no-tillage systems. Figure S2: Correlation matrices among maize performance, soil phosphorus availability,

arbuscular mycorrhizal colonization, rhizosphere biological attributes, and root system architecture under contrasting no-tillage systems. Table S1: Analysis of variance (ANOVA) for maize grain yield (kg ha^{-1}) and resin-extractable P (mg dm^{-3}) in inoculated and non-inoculated maize under 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 and 2022/2023 growing seasons. Table S2: Interaction between phosphorus (P) fertilization and PSB inoculation effects on maize grain yield (kg ha^{-1}) and resin-extractable P (mg dm^{-3}) under 0, 50, and 100% of the recommended P fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 and 2022/2023 growing seasons. Table S3: Analysis of variance (ANOVA) for root system architecture traits of inoculated and non-inoculated maize under 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 growing season. Table S4: Root system architecture traits of inoculated and non-inoculated maize under 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 growing season. Table S5: Analysis of variance (ANOVA) for root system architecture traits of inoculated and non-inoculated maize under 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2022/2023 growing season. Table S6: Root system architecture traits of inoculated and non-inoculated maize under 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2022/2023 growing season. Table S7: Analysis of variance (ANOVA) for the relative abundance (%) of bacterial phyla in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 growing season. Table S8: Analysis of variance (ANOVA) for the relative abundance (%) of bacterial phyla in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2022/2023 growing season. Table S9: Analysis of variance (ANOVA) for the relative abundance (%) of bacterial families in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 growing season. Table S10: Analysis of variance (ANOVA) for the relative abundance (%) of bacterial families in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2022/2023 growing season. Table S11: Analysis of variance (ANOVA) for the Simpson, Shannon, and Chao diversity indices of bacterial families in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 and 2022/2023 growing seasons. Table S12: Simpson, Shannon, and Chao diversity indices of bacterial families in the maize rhizosphere under PSB inoculation and 0, 50, and 100% of the recommended phosphorus (P) fertilization rate in the newly established (NTS-NC) and consolidated (NTS-C) no-tillage systems during the 2021/2022 and 2022/2023 growing seasons.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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