Stronger effect of maize rhizosphere than phosphorus fertilization on soil phosphatase activity and associated bacterial communities in acidic soil: distinct influences on *phoC*- and *phoD*-harboring bacteria

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Abstract

Aims

The bacteria *phoC* and *phoD* genes encode acid and alkaline phosphatase (ACP and ALP), respectively, which mineralize organic phosphorus (P) to inorganic P. The relative importance of P fertilization and the plant rhizosphere on soil phosphatase activities and associated bacterial communities in acidic soils are poorly understood; whether *phoC*- and *phoD*-harboring bacterial communities display different responses remains undetermined.

Methods

Maize was grown in acidic soil supplemented with 0 (P0), 20 (P20), and 200 (P200) mg P₂O₅ kg⁻¹ for 42 days. Maize biomasses, plant nutrients, soil properties, phosphatase activities, and associated bacterial abundance and community composition were determined.

Results

Relative to bulk soils, rhizosphere showed increased ACP and ALP activities, *phoC* and *phoD* gene abundance, but these effects were reduced in strength with P200 treatment, except for *phoC* gene abundance. The rhizosphere effect increased α -diversity of *phoC*-harboring bacteria under P fertilization but reduced α -diversity of *phoD*-harboring bacteria under P0 and P20 treatments. The rhizosphere significantly influenced both *phoC*- and *phoD*-harboring bacterial community compositions, with stronger effect on *phoD*-harboring bacteria; while P fertilization affected *phoD*-harboring bacteria but not *phoC*-harboring bacteria. Immigrated and extinct species play important roles in reshaping *phoC*- and *phoD*-harboring bacterial communities, respectively, in response to the rhizosphere effect.

Conclusions

Compared with P fertilization, the maize rhizosphere more strongly influenced soil phosphatase activities and *phoC*- and *phoD*-harboring bacterial communities in acidic soils, with *phoD*-harboring bacteria responding more strongly to the rhizosphere effect and P fertilization. Notably, the strength of the rhizosphere effect heavily relied on P fertilization level.

Introduction

Phosphorus (P) is a crucial nutrient for plant growth, but its bioavailability is mostly limited in agro-ecosystems (Bi et al. 2020). In traditional agriculture, high input of mineral P fertilizers is continuously applied to obtain a high crop yield. However, the low utilization of P fertilizers results in a large amount of

P being fixed in the soil, which not only causes a waste of P resources but also presents high-risk environmental issues (Gou et al. 2021). As an important part of soil microorganisms, phosphate-solubilizing microorganisms (PSM) are among the main drivers for transforming soil-fixed P into bioavailable forms (Billah et al. 2019). One of the important pathways of P activation mediated by PSM is the secretion of phosphatases, including acid phosphatase (ACP) and alkaline phosphatase (ALP), which can release bioavailable P from soil organic sources (Nannipieri et al. 2011). The *phoC* and *phoD* genes, separately as a part of the phosphate regulon (Pho) in coding for ACP and ALP production, have been used as molecular biomarkers to target PSM communities associated with phosphatase secretion (Fraser et al. 2015; Luo et al. 2019; Tan et al. 2013).

The production of PSM-derived phosphatase is closely correlated with the attributes of PSM communities, including abundance, diversity and composition (Fraser et al. 2017; Luo et al. 2019). These attributes are substantially altered by fertilizer application, a major agricultural practice, which then influences soil phosphatase activities (Luo et al. 2019; Zheng et al. 2021). Among the various fertilization regimes, the effect of P fertilizer on soil PSM communities is particularly concerning because it involves the balance between soil P input and consumption. P fertilizer application has been suggested to exert negative effects on soil PSM populations and phosphatase activities (Chen et al. 2019a); by contrast, its positive (Zheng et al. 2021) or neutral (Hu et al. 2018) effects have also been reported. Among PSM communities, *phoD*-harboring bacteria have been the focus of the numerous studies, whereas *phoC*-harboring bacteria remain rarely studied. Specifically, the effects of fertilization on soil *phoC*- and *phoD*-harboring bacterial communities largely vary across different ecosystems, even within the same habitat (Fraser et al. 2017; Luo et al. 2019; Zheng et al. 2019). For example, Luo et al. (2019) observed that treatment with both organic and chemical fertilizers increased *phoC* gene diversity but did not change *phoD* gene diversity. Long-term fertilization of acidic soil showed that chemical fertilizer input decreased α -diversity of *phoD*-harboring bacteria but exerted no effect on *phoC*-harboring bacteria (Zheng et al. 2021). The inconsistency of PSM community responding to fertilization is likely attributable to differences in ecosystem type (Hu et al. 2018), microbial taxa (Liu et al. 2020; Tan et al. 2013), and type and amount of fertilizer (Chen et al. 2017; Chen et al. 2019b). Whether soil *phoC*- and *phoD*-harboring bacterial communities display different or identical responses to P fertilization is thus far inconclusive.

The rhizosphere is not only the primary site for plant P absorption (Hinsinger, 2001) but is also the hot spot that drives microbial population density and activities (Kuzyakov and Blagodatskaya 2015). The biogeochemistry of this environment is fundamentally altered by the input of root exudates and a decrease in soil nutrient caused by plant growth (Fan et al. 2017). Organic substances produced by plant roots supply large quantities of the available carbon (C) source, which can stimulate PSM to produce copious amounts of phosphatase (Richardson et al. 2009). Meanwhile, the rhizosphere plays an important role in regulating microbial activity for P mining by recruiting specific PSM species (Kuzyakov and Blagodatskaya 2015). Microbial species related to P cycling can be selectively colonized in the rhizosphere via niche filtering, mainly by secreting specific root exudate compounds (Mendes et al. 2014). Inevitably, the strength of the rhizosphere effect on soil microorganisms is affected by fertilization in agriculture because fertilization regimens, such as the type and amount of fertilizer, substantially change

soil properties and plant growth (Lagos et al. 2016; Liu et al. 2020). Zheng et al. (2021) reported that a rise in soil phosphatase activity in response to fertilization is largely attributable to an increase in plant biomass, which can produce more root exudation. In some habitats, the plant rhizosphere more strongly influences soil microbial communities when compared with fertilization (Wang et al. 2020). Lagos et al. (2016) similarly reported on the greater influence of the plant rhizosphere on *phoD*-harboring bacterial community than phosphate and phytate additions. The effects of P fertilization and plant rhizosphere on soil PSM communities and phosphatase activities have been established (Fraser et al. 2017; Lagos et al. 2016; Luo et al. 2019); however, their combined effects and relative strength have not been reported. Further research into this biological process is essential to understand how PSM communities respond to P fertilization in a soil–plant system.

Acidic soil (pH < 5.5) comprises about 22.7% of the total soil area in China and is widely distributed in southern China (Huang and Zhao 2014). P availability in soil is particularly low in this region (Zhao et al. 2013), rendering that soil P deficiency is a major factor limiting crop production and microbial functions (Zheng et al. 2019). As a metabolic phosphatase substrate, soil organic P (P_o) is an important component of the P source in acidic soils (Xiang et al. 2003), and significantly contributes to plant P nutrition (Richardson et al. 2009). Mineralizing P_o by effectively exerting soil PSM function is considered a feasible and environment-friendly approach to enhance plant P nutrient uptake in acidic soils (Billah et al. 2019). Owing to its important role in activating the source of soil P, PSM communities in acidic soils have drawn increased attention (Gou et al. 2021; Zheng et al. 2021). The optimized use of the ability of PSM to mineralize P_o can support specific ecosystem services and allow the reduction of P fertilizer inputs in acidic soils.

Maize (*Zea mays* L.) as an important crop is widely grown globally, including the acidic-soil regions of China. Nutrient deficiencies (particularly P deficiency) in acidic soils frequently hamper maize production (Tandzi et al. 2018). Therefore, the role of soil PSM in acidic soils needs to be utilized to improve the P uptake of maize. In the current study, we hypothesized that the ACP and ALP activities and *phoC*- and *phoD*-harboring bacterial communities in response to both P fertilization and the rhizosphere effects of maize varied in acidic soils, and the rhizosphere played a more important role in reshaping associated bacterial communities when compared with P fertilization. To verify these hypotheses, maize was planted in acidic soil receiving different P fertilizer levels, and soil ACP and ALP activities, *phoC* and *phoD* gene abundance, and associated bacterial communities in bulk and rhizosphere soil samples were determined. This study aimed to (1) determine the relative importance of P fertilization and maize rhizosphere in soil phosphatase activities and PSM population in acidic soil; (2) evaluate the different responses of *phoC*- and *phoD*-harboring bacterial communities to P fertilization and the rhizosphere effect.

Materials And Methods

Soil pot experiment

The acidic soil (0–20 cm) was collected in April 2019 at Yingtan Red Soil Ecological Experiment Station (28° 14' N, 117° 03' E) in Jiangxi Province, China. The soil was air-dried and sieved through a 2 mm mesh after the roots and leaves were removed. The basic properties of soil are listed in Supplementary Table 1.

Plants were grown in plastic pots with dimensions of 175 mm (open top) × 135 mm (height) × 125 mm (flat bottom). Calcium superphosphate was supplied as the P fertilizer, and three P fertilization levels were designed: 0 (P0), 20 (P20), and 200 (P200) mg P₂O₅ kg⁻¹ soil. Urea and potassium sulfate were applied at 88.9 mg N and 33.2 mg K kg⁻¹ soil with P fertilizer as the base fertilizer. Each fertilization treatment had six replicate pots, including three plant pots and three non-planted pots. Basic fertilizer and 2.5 kg of soil were thoroughly mixed in each plastic pot, and the mixed soil was preincubated for 7 days before sowing. All maize seeds (*Zea mays* L., cv. Zhengdan 958) were sterilized with 10% hydrogen peroxide and then rinsed three times with distilled water. Three seeds per pot were sown directly into the soil. Soil moisture was maintained at 60% field capacity, and all pots were placed in a natural greenhouse.

Sampling and plant nutrient analysis

Plants were harvested after 42 days of sowing. All plant roots were separated from the soil in each pot. The soil that adhered to the roots was defined as rhizosphere soil and collected by shaking manually to remove loosely attached soil. The rhizosphere soil in the same pot was pooled into a single composite sample. The soil samples within the non-plant pots were collected as bulk soil. Each soil sample was mixed by passing it through a 2 mm sieve and divided into three subsamples. One subsample was stored at -20 °C for DNA extraction, and another subsample was stored at 4 °C to analyze soil phosphatase activities, ammonium nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N) within one week; the remaining soil samples were air-dried to measure other soil properties.

Shoots and roots were washed three times using deionized water. Subsequently, all samples were dried at 85 °C in an oven to a constant weight. The biomass of the shoots and roots was measured using an analytical balance, and the shoots were ground (< 1.0 mm) to determine the concentration of each nutrient (N, P and K). The ground shoot samples were digested with H₂SO₄-H₂O₂. N and P concentrations were determined by the methods of Kjeldahl (Lu 1999) and Bray (Bray and Kurtz 1945), respectively. K concentration was measured by flame photometry (FP640, Shanghai, China).

Determination of soil properties and phosphatase activities

Fresh soil was extracted by shaking it with 2.0 M KCl to determine NH₄⁺-N and NO₃⁻-N by continuous flow analysis (San⁺⁺, Skalar, Holland). After the water-and-soil mixture (1: 2.5 w/v) was shaken, the soil pH was determined by pH meter (Mettler Toledo FE20, Shanghai, China). Soil organic C (SOC) was measured by K₂Cr₂O₇ oxidation (Sims and Haby 1971). Soil total N (TN) was measured using a Vario MAX CNS elemental analyzer (Elementar, Hanau, Germany). After the soil was digested with H₂SO₄-HClO₄, total P (TP) and total K (TK) concentrations were determined using the Bray method (Bray and Kurtz 1945) and

by flame photometry (FP640, Shanghai, China), respectively. Available P (AP) was extracted with 0.03 M ammonium fluoride–hydrochloric acid and then measured according to the molybdenum blue method (Lu 1999). Available K (AK) was extracted with 1.0 M ammonium acetate at pH 7.0 and then determined by flame photometry (FP640, Shanghai, China). Soil ACP and ALP activities were determined using the Tabatabai and Bremner method (Tabatabai and Bremner 1994).

DNA extraction and quantification of gene abundance

Soil total DNA was extracted from 0.5 g of fresh soil by using the Fast DNA SPIN Kit (MP Biomedicals, CA, USA). DNA was subsequently purified using the PowerClean DNA Cleanup Kit (Mobio, Carlsbad, CA, USA). The quality and quantity of the purified DNA were determined by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA) and then stored at -20 °C for further use.

The abundances of the *phoC* and *phoD* genes were measured by quantitative polymerase chain reaction (qPCR) on a LightCycler 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany) with SYBR®Premix Ex Taq™ (TaKaRa Bio, Dalian, China) by using the universal primers *phoC*-A-F1 (5'-CGGCTCCTATCCGTCCGG -3')/*phoC*-A-R1 (5'-CAACATCGCTTTGCCAGTG -3') (Fraser et al. 2017) and ALPS-F730 (5'-CAGTGGGACGACCACGAGGT -3')/ALPS-R1101 (5'-GAGGCCGATCGGCATGTCTG -3') (Tan et al. 2013). The qPCR process, reaction composition and standard curves were described in Zheng et al. (2019). Three replicates for each DNA sample were determined. The PCR amplification efficiencies of *phoC* and *phoD* genes were 96.88% and 96.66%, respectively; and both R^2 values of the standard curve exceeded 0.98.

High-throughput sequencing and data processing

As already described, each DNA sample was amplified with *phoC* and *phoD* gene primers, respectively. Sample-specific 7 bp barcodes were incorporated into the forward primer to differentiate the sample. Three replicates were amplified for each DNA sample, and their PCR products were pooled as one single sample. The purified amplicons were then pooled in equimolar concentrations, and paired-end sequencing was conducted using Illumina HiSeq PE150 and Illumina Miseq PE250 for the *phoC* and *phoD* genes, respectively (Shanghai Personal Biotechnology Co., Ltd.). The sequencing data of the *phoC* and *phoD* genes were deposited in the NCBI Sequence Read Archive (SRA) database (accession number: SRP332162 for *phoC* gene and SRP332164 for *phoD* gene).

Pairs of reads from the raw data were first merged with FLASH (version 1.2.7) (Magoc and Salzberg 2011), and sequencing reads were processed with the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) pipeline (Caporaso et al. 2010). Low-quality sequences with lengths of < 130 bp for *phoC* and < 150 bp for *phoD* and sequences containing ambiguous nucleotides and not matching the primer were eliminated. USEARCH (version 5.2.236) were used to examine and remove chimeric sequences. After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by UCLUST (Edgar 2010). The sequence, which was the most abundant

sequence in each OTU, was identified using the closest relative from a BLAST algorithm-based search within GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Each sample was rarefied to the identical number of *phoC* and *phoD* reads (17,412) to ensure an even sampling depth for community analysis.

Statistical analysis

The α -diversity indices including richness and Shannon index were calculated using QIIME. Significant differences in the plant biomass and nutrient (N, P and K) concentrations of the plant shoots among treatments were tested using one-way analysis of variance (ANOVA) with Duncan's tests in SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Two-way ANOVA was conducted to analyze the effects of P fertilization and sampling sites (bulk and rhizosphere) on soil properties, phosphatase activities, gene copy numbers, the relative abundance of genera, niche breadth and α -diversity indices. If the differences were statistically significant, one-way ANOVA or *t*-tests were performed to analyze homogeneity of variance.

To visualize the community similarities of *phoC*- and *phoD*-harboring bacteria among the samples, a principal coordinate analysis (PCoA) based on the Bray–Curtis distance was performed using the “vegan” package in the software R (version 4.0.2). Permutational multivariate analysis of variance (PERMANOVA) was conducted to analyze the effects of P fertilization and sampling sites on *phoC*- and *phoD*-harboring bacterial community compositions. Bray–Curtis dissimilarity analysis was applied to reveal the community dissimilarity between bulk and rhizosphere soil samples for each P fertilizer level and between each P fertilizer (P20 or P200) and P0 (no P fertilizer) for the bulk or rhizosphere soil samples. The contribution of the dominant genus to the community variations was quantified using similarity percentage (SIMPER) analysis. The Mantel test was conducted to identify the correlations between the bacterial community structure and soil properties. PERMANOVA, the Bray–Curtis dissimilarity, SIMPER and the Mantel test were conducted using the “vegan” package in R. The niche breadth of the bacterial community in each sample was analyzed using the “spaa” package in R.

To evaluate the effects of species turnover in shaping rhizosphere bacterial communities, OTUs were divided into three parts: OTUs detected in bulk soil samples but not in the rhizosphere (extinction), OTUs detected in the rhizosphere but not in the bulk soil samples (immigration), and OTUs detected in both the bulk and rhizosphere soil samples (share). The contributions of shared, immigrated and extinct OTUs to the variations in richness and community composition were expressed as proportion and relative abundances, respectively. The proportion was the percentage of the shared, immigrated and extinct OTUs account for the total OTU richness that included these three parts. The relative abundance was calculated using the ratio of the shared, immigrated and extinct OTUs sequences to total gene sequences, respectively. The contributions of the shared, immigrated and extinct OTUs to the community dissimilarity were calculated using the method reported by Hillebrand et al. (2018):

$$BC_{jk} = \frac{\sum |X_{ij} - X_{ik}|}{\sum |X_{ij} + X_{ik}|} \quad (1)$$

$$BC_{jk(S/I/E)} = \frac{\sum |X_{(S/I/E)j} - X_{(S/I/E)k}|}{\sum |X_{(S/I/E)j} + X_{(S/I/E)k}|} \quad (2)$$

$$Con_{(S/I/E)} = \frac{BC_{jk(S/I/E)}}{BC_{jk}} \quad (3)$$

where BC is the Bray–Curtis dissimilarity between bulk soil samples (j) and rhizosphere soil samples (k); X is the gene sequences of the taxon i or S, I, E , respectively, where i represents each taxon, and S, I , and E refer to the shared, immigrated and extinct taxon, respectively. Con denotes the contribution of the shared, immigrated and extinct OTUs to bacterial community dissimilarity, respectively.

Using the aforementioned calculation method, we also determined the contributions of the shared, immigrated and extinct OTUs to the variations in richness, community composition and dissimilarity between each P fertilizer (P20 or P200) and P0 in the bulk or rhizosphere soil samples. Extinct OTUs indicate the OTUs detected under the P0 treatment but not in P fertilizer, and immigrated OTUs represent the OTUs detected in P fertilizer but not under the P0 treatment. Shared OTUs denote the OTUs detected in both P0 and P20 or P200.

Results

Maize growth and soil physicochemical properties

Maize biomass (shoot and root) was markedly higher under the P200 treatment ($p < 0.05$) than the P0 and P20 treatments (Fig. 1a). P fertilizer application (P20 and P200) significantly increased ($p < 0.05$) the plant P concentrations but decreased the K concentrations in the shoot (Fig. 1b). The plant N concentrations in the shoot showed no significant differences among treatments.

Two-way ANOVA showed that the P fertilizer supply significantly influenced ($p < 0.05$) soil pH, the contents of SOC, NH_4^+ -N, NO_3^- -N, AP and TP, and the C:P and N:P ratios. The sampling sites (bulk and rhizosphere) markedly affected ($p < 0.05$) the contents of SOC, NH_4^+ -N, NO_3^- -N, AP and AK, and the C:N and C:P ratios (Table S2). P fertilizer application (P20 and P200) reduced the NH_4^+ -N and NO_3^- -N contents but increased the AP and TP contents (Table S2). The NH_4^+ -N, NO_3^- -N and AK contents in the rhizosphere soil samples under P fertilizer application (P20 and P200) were lower ($p < 0.05$) than those in bulk soil samples, whereas the C:N ratio exhibited the opposite trend (Table S2).

Soil Phosphatase Activities And Gene Abundance

Two-way ANOVA indicated that both P fertilization and sampling sites significantly influenced ($p < 0.05$) the activities of soil ACP and ALP and the abundances of the *phoC* and *phoD* genes, however, sampling sites exerted stronger effects than did P fertilization (Fig. 2). Compared with P0, P200 markedly decreased ($p < 0.05$) the ACP and ALP activities, except for ALP activities in the rhizosphere (Figs. 2a and

2b). For each treatment, the ACP and ALP activities in the rhizosphere were statistically higher than those in the bulk soil samples, except for ALP activities in the P0 treatment (Figs. 2a and 2b). Compared with P0, P200 showed higher ($p < 0.05$) *phoC* gene abundance in the rhizosphere but exhibited no significant effect on *phoD* gene abundance in the rhizosphere (Figs. 2c and 2d). Relative to the bulk soil samples, the rhizosphere soil sample from each treatment exhibited increased *phoC* and *phoD* gene abundances; meanwhile only the *phoC* gene abundance of the P200 samples and the *phoD* gene abundance of both the P0 and P20 samples showed statistical differences (Figs. 2c and 2d).

Community composition and diversity of *phoC*- and *phoD*-harboring bacteria

The dominant genera ($> 0.5\%$) for the *phoC*-harboring bacterial community contained *Shimwellia*, *Klebsiella*, *Xanthomonas*, *Stenotrophomonas*, *Cupriavidus* and *Bradyrhizobium* (Fig. 3a). The dominant genera for the *phoD*-harboring bacterial community contained *Collimonas*, *Bradyrhizobium*, *Streptomyces*, *Pseudomonas*, *Cupriavidus*, *Halomonas*, *Actinoplanes*, *Amycolatopsis*, *Kitasatospora*, *Pleomorphomonas* and *Roseovarius* (Fig. 3b). A two-way ANOVA test showed that P fertilization significantly influenced ($p < 0.05$) the relative abundance of *Shimwellia* (*phoC*-harboring bacteria, Table S3), whereas both P fertilization and sampling sites affected ($p < 0.05$) the relative abundance of *Collimonas*, *Bradyrhizobium* and *Kitasatospora* (*phoD*-harboring bacteria, Table S4). In addition, sampling sites influenced ($p < 0.05$) the relative abundance of *Pseudomonas*, *Halomonas*, *Amycolatopsis* and *Pleomorphomonas* (*phoD*-harboring bacteria, Table S4).

A two-way ANOVA test showed that the sampling sites markedly affected ($p < 0.01$) the richness and Shannon values of *phoC*- and *phoD*-harboring bacterial communities, and P fertilization influenced ($p < 0.01$) the richness and Shannon values of the *phoD*-harboring bacterial community but not those of the *phoC*-harboring bacteria (Figs. 3c–3f). The rhizosphere soils showed higher richness and Shannon values of the *phoC*-harboring bacterial community than in the bulk soil samples under the P20 and P200 treatments (Figs. 3c and 3e). However, the richness and Shannon values of the *phoD*-harboring bacterial community were significantly lower ($p < 0.01$) in the rhizosphere than in the bulk soil samples under the P0 and P20 treatments (Figs. 3d and 3f). The richness and Shannon values of the *phoD*-harboring bacterial community in the rhizosphere samples were increased ($p < 0.05$) under the P200 treatment, relative to the P0 and P20 treatments.

A two-way ANOVA test showed that the sampling sites significantly affected ($p < 0.05$) the niche breadth of both *phoC*- and *phoD*-harboring bacteria, whereas P fertilization exerted no such effect (Fig. 4). P fertilization treatments (P20 and P200) increased the niche breadths of the *phoC*-harboring bacteria in the rhizosphere relative to those in the bulk soil samples, but a significant difference was found only under the P200 treatment ($p < 0.05$, Fig. 4a). However, under both P0 and P20 treatments, the *phoD*-harboring bacteria showed a lower niche breadth ($p < 0.05$) in the rhizosphere than in the bulk soil samples (Fig. 4b).

phoC - and *phoD*-harboring bacterial community structures

The PCoA results revealed that both *phoC*- and *phoD*-harboring bacterial communities were separated among soil samples (Figs. 5a and 5b). PERMANOVA results indicated that the sampling sites significantly affected ($p < 0.05$) both *phoC*- and *phoD*-harboring bacterial community compositions, whereas P fertilization influenced ($p < 0.05$) the *phoD*-harboring bacterial community composition but not the *phoC*-harboring bacterial community (Fig. 5c). Moreover, the influence of the sampling sites on the *phoD*-harboring bacterial community composition ($F = 11.889$, $p = 0.001$) was greater than that of P fertilization ($F = 3.060$, $p = 0.015$; Fig. 5c). With an increase in the supply of P fertilizers, the dissimilarities in the *phoC*-harboring bacterial communities between the bulk and rhizosphere soil samples significantly increased ($p < 0.05$, Fig. 6a). The dissimilarity in the *phoD*-harboring bacterial communities between the bulk and rhizosphere soil samples was increased under the P20 treatment relative to the P0 treatment but was markedly decreased under the P200 treatment (Fig. 6b). In addition, P fertilizer supply did not affect the *phoC*-harboring bacterial community dissimilarities in the bulk and rhizosphere soil samples (Fig. 6c); meanwhile, a high dissimilarity in the *phoD*-harboring bacterial communities in the rhizosphere soils was found between the P200 and P0 treatments (Fig. 6d). The Mantel test indicated that the *phoD*-harboring bacterial community structure was related ($p < 0.05$) to variations in soil pH, SOC, C:N ratio and C:P ratio; by contrast, no significant correlation was found between the *phoC*-harboring bacterial community structure and soil variables (Table S5).

SIMPER analysis indicated that *Klebsiella* and *Shimwellia* mostly contributed to the variations in the *phoC*-harboring bacterial community between the bulk and rhizosphere soil samples under each treatment, and *Xanthomonas* showed a high contribution under the P200 treatment (Table 1). Both *Collimonas* and *Bradyrhizobium* largely contributed to variations in the *phoD*-harboring bacterial community between the bulk and rhizosphere soil samples under the P0 and P20 treatments, and *Streptomyces* and *Collimonas* showed large contributions under the P200 treatment (Table 1). In addition, both *Klebsiella* and *Shimwellia* contributed mostly to the *phoC*-harboring bacterial community variations among treatments in the bulk and rhizosphere soils samples (Table S6). Similarly, *Collimonas* and *Bradyrhizobium* largely contributed to variations in the *phoD*-harboring bacterial community (Table S6).

Table 1
Respective contributions of each dominant genus to variations in *phoC*- and *phoD*-harboring bacterial communities between bulk and rhizosphere soil samples for each treatment, as determined by SIMPER analysis.

	Genus	P0	P20	P200
<i>phoC</i>	<i>Klebsiella</i>	5.740	8.902	12.547
	<i>Shimwellia</i>	4.862	5.000	3.672
	<i>Stenotrophomonas</i>	1.200	1.831	0.479
	<i>Cupriavidus</i>	0.982	0.440	0.184
	<i>Xanthomonas</i>	0.272	0.403	4.355
	<i>Bradyrhizobium</i>	0.221	0.163	0.186
<i>phoD</i>	<i>Collimonas</i>	29.493	38.919	3.886
	<i>Bradyrhizobium</i>	13.307	13.059	3.055
	<i>Streptomyces</i>	4.573	7.095	4.826
	<i>Pseudomonas</i>	3.377	3.000	1.724
	<i>Pleomorphomonas</i>	0.728	0.352	0.201
	<i>Actinoplanes</i>	0.635	0.687	0.689
	<i>Halomonas</i>	0.543	0.340	0.391
	<i>Cupriavidus</i>	0.506	3.439	1.236
	<i>Kitasatospora</i>	0.430	0.792	0.180
	<i>Amycolatopsis</i>	0.182	0.997	0.265

Contributions of species turnover in shaping *phoC*- and *phoD*-harboring bacterial communities

Compared with bulk soil samples, the rhizosphere showed a higher proportion of immigrated *phoC*-harboring bacterial OTUs, which was observed under the P20 and P200 treatments (Fig. 7a). The increased contributions to the relative abundance and dissimilarity of immigrated *phoC*-harboring bacterial OTUs were found under the P200 treatment (Figs. 7c and 7e). For *phoD*-harboring bacteria, the contributions of extinct OTUs in the rhizosphere to bacterial richness, relative abundance and dissimilarity were higher under the P0 and P20 treatments than the P200 treatment (Figs. 7b, 7d and 7f). However, the shared *phoD*-harboring bacterial OTUs under the P200 treatment contributed more to the total richness, relative abundance and dissimilarity (Figs. 7b, 7d and 7f).

A similar analysis of the effect of P fertilization was conducted (Fig. S1). The bulk and rhizosphere soil samples showed inconsistent responses to P fertilization. The contributions of immigrated OTUs to the richness, relative abundance and dissimilarity of *phoC*-harboring bacteria were higher than those of extinct OTUs in the rhizosphere, whereas the opposite results were observed in bulk soil samples under the P20 treatment (Figs. S1a, S1c and S1e). The contributions of immigrated OTUs to the richness, relative abundance and dissimilarity of *phoD*-harboring bacteria under the P200 treatment were higher than those of extinct OTUs in the rhizosphere, whereas those of extinct OTUs were lower in the rhizosphere than in the bulk soil samples (Figs. S1b, S1d and S1f).

Discussion

Previous studies mainly concentrate on the effects of P fertilization on soil phosphatase activity and the PSM community coding phosphatase (Bi et al. 2020; Luo et al. 2019; Tan et al. 2013) and rarely investigated the plant rhizosphere effect, particularly the effect on *phoC*-harboring bacteria. The variations in community and function of PSM that are regulated and stimulated by the plant rhizosphere have significant implications for plant P nutrition (Billah et al. 2019). Our results clearly suggested that in acidic soils, the maize rhizosphere exerted stronger effects on soil phosphatase activities and *phoC*- and *phoD*-harboring bacterial community attributes (abundance, diversity and composition) than P fertilization (Figs. 2, 3 and 5); meanwhile, the strength of the rhizosphere effect was highly dependent on P fertilization level. This study clarified the relative importance and possible microbial mechanisms underlying the rhizosphere effects and P fertilization on *phoC*- and *phoD*-harboring bacterial communities, which can provide a valuable understanding to effectively fulfill the potential of PSM.

Increased activities of soil phosphatase in the rhizosphere

Our results revealed higher activity for ACP than ALP (Figs. 2a and 2b), which was consistent with Zheng et al. (2019), indicating that ACP played a more important role than ALP in transforming organic P into utilizable form in acidic soils. In the current study, the maize rhizosphere soil samples exhibited higher ACP and ALP activities, compared with the bulk soil samples regardless of P fertilization (Figs. 2a and 2b). This observation was consistent with previous findings, suggesting that soil phosphatase was markedly enriched in the rhizosphere (Kuzyakov and Razavi 2019). Such a pattern is primarily attributed to easily degradable organic compounds from roots, which prompted the stimulation of rhizosphere PSM, and/or the direct release of phosphatase from plant roots (Kuzyakov and Razavi 2019). Although the rhizosphere significantly increased phosphatase activity, this strength decreased in response to a high level of P fertilization—that is, high P fertilization (P200) clearly lowered the rhizosphere phosphatase activities (Figs. 2a and 2b). The reason is that a higher AP concentration in soil inhibits phosphatase synthesis (Gou et al. 2021). Similarly, Liu et al. (2020) found that high-P fertilizer input decreased wheat rhizosphere ALP activity in loess soil with a deficiency in available P. Hofmann et al. (2016) also reported that P fertilizer inputs decreased ACP activity at low-P forest sites. Thus, although root exudates can improve soil phosphatase activity by supplying nutrients to soil microorganisms, phosphatase activity is also partly regulated by negative feedback from high P fertilization.

Different responses of *phoC* and *phoD* gene abundance to rhizosphere effects

Positive correlations between PSM abundance and phosphatase activities in various habitats have been frequently observed (Chen et al. 2017; Zheng et al. 2019). However, in another study, *phoD* gene abundance did not contribute to a change in phosphatase activity in upland red soil (Tian et al. 2021). Fraser et al. (2017) observed the significant positive correlation between *phoD* gene abundance and ALP activity but not between *phoC* gene abundance and ACP activity in the rhizosphere. These differences were attributed to plant type (Ragot et al. 2016), microbial group (Zheng et al. 2021), and fertilization regimes (Luo et al. 2019). In the current study, P fertilization did not affect both *phoC* and *phoD* gene abundances in bulk soil samples (Figs. 2c and 2d), which was in line with the study by Luo et al. (2019). Similar to other reports (Fraser et al. 2017; Zheng et al. 2020), the results of present study indicated that the rhizosphere effect increased soil *phoC* and *phoD* gene abundances to varying degrees (Figs. 2c and 2d). This finding is closely related to root exudates stimulating the growth of microbial species (Fraser et al. 2017). Notably, this effect pattern heavily depended on the P fertilization level and PSM type. Specifically, the increased effect of the rhizosphere on *phoC* gene abundance was statistically significant only under a high P fertilizer input (P200), implying that the *phoC* gene abundance was not the primary contributor to the improvement in ACP activity in the rhizosphere. The rhizosphere effect might contribute to the reproductive growth, rather than the functional performance, of *phoC*-harboring bacterial species after exposure to high-P fertilization. Contrary to *phoC* gene abundance, *phoD* gene abundance significantly increased in the rhizosphere under low-P conditions (P0 and P20) but not under the P200 treatment (Fig. 2d). The rhizosphere environment with a higher C source and lower AP contents (Table S2) might be more conducive to the growth of specific *phoD*-harboring bacteria (Tian et al. 2021). However, the high AP content in the soil, resulting from P fertilization, can cause the negative feedback mechanism for limiting the growth of *phoD*-harboring bacteria (Chen et al. 2019a).

Different rhizosphere effects on *phoC* and *phoD* gene diversity

Similar to gene abundance, the α -diversity (Shannon index and richness) of *phoC*- and *phoD*-harboring bacterial communities exhibited different response patterns to the rhizosphere effect—even opposite trends (Fig. 3). P20 and P200 treatments clearly increased *phoC* gene α -diversity in the rhizosphere, which could improve the probability of including taxa adapted to specific abiotic conditions (Kurm et al. 2019). The reason could be that the improved maize biomasses after P fertilizer application supplied more root exudates to the rhizosphere environment. Diverse components of exudates can provide an appropriate condition for different types of microbial taxa (Kuzyakov and Blagodatskaya 2015). Specifically, rare microbial taxa as reservoirs of genetic resources are difficult to find because of their low abundance; meanwhile, they may be activated under appropriate conditions and even become dominant species (Zhang et al. 2018). In the present study, immigrated OTUs contributed more to the richness and relative abundance of *phoC*-harboring bacteria in the rhizosphere treated with P fertilizers, particularly the P200 treatment (Figs. 7a and 7c). Under controlled conditions, newly detected taxa may be derived from low-abundance taxa in the original inoculum (Jiao et al. 2017). Thus, the specific rhizosphere environment

under P fertilization may activate some low-abundance *phoC*-harboring bacterial species and promote their growth.

By contrast, the α -diversity of the *phoD*-harboring bacterial community was significantly lower in the rhizosphere than in the bulk soil samples under the P0 and P20 treatments (Figs. 3d and 3f). This result suggested that the rhizosphere effect on *phoD*-harboring bacteria mainly exhibited selection rather than immigration, as verified by the lower niche breadth of *phoD*-harboring bacteria in the rhizosphere (Fig. 4b) and higher contribution of extinct *phoD*-harboring bacterial OTUs to variations in total richness and relative abundance (Figs. 7b and 7d). Similarly, Liu et al. (2021) reported that the maize rhizosphere showed the reduced *phoD* gene diversity in P-deficient Karst soil. The selective pressure in the rhizosphere with low P status would lead to a change in bacterial community composition associated with fitness-based species selection (Mander et al. 2012). This finding implies that *phoD*-harboring bacteria may have a strict requirement for a rhizosphere environment. For instance, specific *phoD*-harboring genus is more preferentially selected under P-poor and C-rich conditions (Tian et al. 2021). The growth of some *phoD*-harboring bacterial species, such as *Lysobacter antibioticus* and *Bradyrhizobium icense*, was inhibited in the rhizosphere (Liu et al. 2021). However, once the specific taxa were selected, they grow rapidly in the rhizosphere. This observation was confirmed by the higher relative abundance of some *phoD*-harboring bacterial genera in the rhizosphere (Fig. 3b). The *phoD*-harboring bacterial taxa selected in the maize rhizosphere would show an important role in determining ALP activity (Liu et al. 2021). Notably, high P fertilization (P200) showed no selective pressure of the rhizosphere on *phoD*-harboring bacteria (Figs. 3d and 3f). High fertilizer inputs decrease the difficulty of organisms to obtain nutrients (Sun et al. 2020). This reduction may alleviate niche selection by lessening the dependence of PSM on P_o mineralization and create a more on-limits habitat with a wider niche breadth for *phoD*-harboring bacteria to provide improved protection against the loss of taxa (Mander et al. 2012; Sun et al. 2020). This result reflects the capacity of *phoD*-harboring bacteria for ecological niche preservation under high P fertilization.

Distinct variations in *phoC*- and *phoD*-harboring bacterial community composition in response to rhizosphere effects

Previous studies have revealed the significant effect of rhizosphere on *phoD*-harboring bacterial community composition (Liu et al. 2021; Mander et al. 2012). Root exudates and soil nutrient levels in the rhizosphere are considered primary factors driving the variation in *phoD*-harboring bacterial community (Gou et al. 2021; Mander et al. 2012). In the current study, the rhizosphere effect markedly influenced both *phoC*- and *phoD*-harboring bacterial community compositions, although the *phoC*-harboring bacterial community exhibited a weak response relative to the *phoD*-harboring bacterial community (Fig. 5). On the one hand, ACP production by plants and microbes is well established (Nannipieri et al. 2011), and ACP produced by plant root can reportedly negate the effects of the *phoC*-harboring bacterial community in the rhizosphere (Fraser et al. 2017). On the other hand, although soil variables play an important role in structuring microbial communities in the rhizosphere (Fan et al. 2017), the *phoC*-harboring bacterial community was not as sensitive to soil variables as the *phoD*-harboring bacteria community (Table

S5). The dominant *phoC*-harboring bacterial genera, including *Shimwellia* and *Klebsiella* (Fig. 3a), were considered as oligotrophic taxa (Starke et al. 2016). These oligotrophic taxa often show the reduced growth rates and may exhibit the decreased nutrient demands (Yang et al. 2020), resulting in insensitivity to variations in soil variables in the rhizosphere.

The strength of the rhizosphere effect on PSM community composition also relied on the level of P fertilizer, particularly for the *phoD*-harboring bacteria (Fig. 5b). As oligotrophic bacteria, dominant *phoC*-harboring bacteria exposed to environmental gradients may maintain viability and stability (Fierer et al. 2007). Therefore, the *phoC*-harboring bacterial community may only slightly change in response to P fertilization. In the current study, low P fertilization (P0 and P20) relative to high P input (P200) markedly increased the selective effect of the rhizosphere on taxa for the *phoD*-harboring bacteria (Figs. 3b). The change in community composition under low P fertilization was attributable to the selection and stimulation of the rhizosphere environment (C-rich and P-poor conditions) for specific *phoD*-harboring bacterial taxa, as previously mentioned (Fig. 7f). Mander et al. (2012) found that low P in soils induced selective pressure for *phoD*-harboring bacteria. However, a less selective effect may be observed under rich-nutrient conditions (Bao et al. 2020). High-P fertilizer input increases compositional stochasticity by stimulating microbial growth (particularly rare taxa), weakening the niche selection for *phoD*-harboring bacteria by reducing competition for resources (Sun et al. 2020). This finding was confirmed by the low Brady–Curtis dissimilarity between the bulk and rhizosphere soil samples under the P200 treatment in the current study (Fig. 6b).

The rhizosphere *phoC*- and *phoD*-harboring bacterial genera that contributed to the increase in soil phosphatase activities also strongly depended on P fertilization levels. Compared with the bulk soil samples, the rhizosphere enriched the *phoC*-harboring genus *Stenotrophomonas* under low-P fertilization (P0 and P20) (Fig. 3a). *Stenotrophomonas* was identified as the important contributor of ACP production in acidic soils and regarded as the keystone species in the co-occurrence network of the *phoC*-harboring bacterial community (Zheng et al. 2021). With high-P fertilizer input (P200), the maize rhizosphere exhibited higher relative abundance of the *phoC*-harboring genus *Xanthomonas* (Fig. 3a). *Xanthomonas* was identified as the keystone taxon in the *phoC*-harboring bacterial community after long-term fertilization application (Luo et al. 2019). Notably, unclassified OTUs were more enriched in the rhizosphere under the P fertilization treatments (P20 and P200) (Fig. 3a), indicating that some unidentified genera could participate in ACP production. The *phoC* primer used in the current study targeted the dominant taxonomic groups but might not necessarily cover the full range of potential *phoC* gene sequences in soils (Gaiero et al. 2018).

Compared with the bulk soil samples, the maize rhizosphere markedly enriched more species of the *phoD*-harboring genera *Collimonas* under the P0 and P20 treatments (Fig. 3b), indicating that *Collimonas* should be effectively selected and better fit to the rhizosphere environment under P-deficient conditions. *Collimonas* has been reported to be among the dominant *phoD*-harboring bacteria and is an important ALP producer (Wang et al. 2021; Zheng et al. 2021). *Collimonas* can strongly assimilate root-derived C to stimulate their growth (Ai et al. 2015) and shows great ability to dissolve P_i in soils (Leveau

et al. 2010). Therefore, *Collimonas* potentially enhance the availability of soil P by mineralizing P_o and solubilizing P_i simultaneously.

Conclusion

The present study revealed that although both the maize rhizosphere and P fertilization collectively affected the attributes and function of the PSM community in acidic soils, the rhizosphere exerted a stronger effect than P fertilization. High phosphatase activities in the rhizosphere suggested the high potential of PSM mineralizing P_o . The maize rhizosphere exerted different effects on *phoC*- and *phoD*-harboring bacterial communities, and the strength of which strongly depended on the P fertilization level. Compared with *phoC*-harboring bacteria, *phoD*-harboring bacteria were more sensitive to the rhizosphere effect. The community assemblies of the *phoC*- and *phoD*-harboring bacteria in response to the rhizosphere effect were mainly reflected in the immigration and filtering effect for species, respectively. These findings indicate that the rhizosphere effect should be more seriously considered when PSM strains are to be used as plant inoculants.

Declarations

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Figures

Figure 1

Biomass (a), and shoot N, P and K concentrations (b) of maize after cultivation with different P fertilizer levels for 42 days. Values presented are the mean $\pm$ SD of three pot replicates. Different letters above the same color columns indicate significant differences ($p < 0.05$) among treatments.

Figure 2

ACP activity (a), ALP activity (b), and *phoC* (c) and *phoD* (d) gene copy numbers in the bulk and rhizosphere soil samples of maize after cultivation with different P fertilizer levels for 42 days. Values presented are the mean $\pm$ SD of three pot replicates. All data were first subjected to two-way ANOVA. P: P fertilizer; S: sampling site (bulk and rhizosphere). Different letters above the same color columns indicate significant differences ($p < 0.05$) among treatments. Asterisk indicates significant difference (* $p < 0.05$ or ** $p < 0.01$) between bulk and rhizosphere soil samples.

Figure 3

Relative abundance of dominant phoC-(a) and phoD-(b) harboring bacterial genera (> 0.5%) in the bulk and rhizosphere soil samples under the different P fertilizer levels. B: bulk soil; R: rhizosphere soil. Richness and Shannon indices of phoC- (c, e) and phoD- (d, f) harboring bacterial communities in the bulk and rhizosphere soil samples under the different P fertilizer levels. Values presented are the mean $\pm$ SD of three pot replicates. All data were first subjected to two-way ANOVA. P: P fertilizer; S: sampling site

(bulk and rhizosphere). Different letters above the same color columns indicate significant differences ($p < 0.05$) among treatments. Asterisk indicates significant difference (* $p < 0.05$ or ** $p < 0.01$) between bulk and rhizosphere soil samples.

Figure 4

Niche breadth of phoC-(a) and phoD-(b) harboring bacteria in bulk and rhizosphere soil samples among treatments. Values presented are the mean ± SD of three pot replicates. All data were first subjected to

two-way ANOVA. P: P fertilizer; S: sampling site (bulk and rhizosphere). Different letters above the same color columns indicate significant differences ($p < 0.05$) among treatments. Asterisk indicates significant difference (* $p < 0.05$ or ** $p < 0.01$) between bulk and rhizosphere soil samples.

Figure 5

Principal coordinate analysis of *phoC*-(a) and *phoD*-(b) harboring bacterial communities, and permutational multivariate analysis of variance (PERMANOVA) (c) among treatments. Black and red dots represent bulk and rhizosphere soil samples, respectively. B: bulk soil; R: rhizosphere soil.

Figure 6

Bray–Curtis dissimilarity values of phoC-(a) and phoD-(b) harboring bacterial communities between bulk and rhizosphere soil samples for each treatment. Bray–Curtis dissimilarity values of phoC-(c) and phoD-(d) harboring bacterial communities between P fertilizer treatment (P20 or P200) and P0 in bulk and rhizosphere soil samples, respectively. Different letters above the columns indicate significant differences ($p < 0.05$) among treatments. Asterisk indicates significant difference ($p < 0.05$) between P20 and P200 samples.

Figure 7

Contributions of shared, immigrated and extinct OTUs to the richness, the relative abundance and community dissimilarity of phoC-(a, c, e) and phoD-(b, d, f) harboring bacterial OTUs between the rhizosphere and bulk soil samples for each treatment.

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