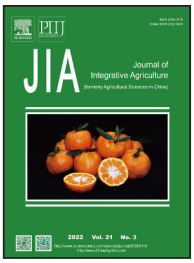
Journal of Integrative Agriculture 2022, 21(3): 840–854

Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

Interactions between phosphorus availability and microbes in a wheat–maize double cropping system: A reduced fertilization scheme

YU Xiao-jing[1], CHEN Qi[1], SHI Wen-cong[1], GAO Zheng[1], SUN Xiao[1], DONG Jing-jing[1], LI Juan[2], WANG Heng-tao[3], GAO Jian-guo[3], LIU Zhi-guang[1], ZHANG Min[1]

[1] *National Engineering Research Center for Efficient Utilization of Soil and Fertilizer Resources/College of Resources and Environment/College of Life Sciences, Shandong Agricultural University, Tai'an 271018, P.R.China*

[2] *Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture and Rural Affairs/Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China*

[3] *Shandong Zhongxin Agricultural Development Co., Ltd., Linshu 276700, P.R.China*

Abstract

Mechanisms controlling phosphorus (P) availability and the roles of microorganisms in the efficient utilization of soil P in the wheat–maize double cropping system are poorly understood. In the present study, we conducted a pot experiment for four consecutive wheat–maize seasons (2016–2018) using calcareous soils with high (30.36 mg kg^{-1}) and low (9.78 mg kg^{-1}) initial Olsen-P content to evaluate the effects of conventional P fertilizer application to both wheat and maize (Pwm) along with a reduced P fertilizer application only to wheat (Pw). The microbial community structure along with soil P availability parameters and crop yield were determined. The results showed that the Pw treatment reduces the annual P input by 33.3% without affecting the total yield for at least two consecutive years as compared with the Pwm treatment in the high Olsen-P soil. Soil water-soluble P concentrations in the Pw treatment were similar to those in the Pwm treatment at the 12-leaf collar stage when maize requires the most P. Furthermore, the soil P content significantly affected soil microbial communities, especially fungal communities. Meanwhile, the relative abundances of Proteobacteria and alkaline phosphatase (ALP) activity of Pw were significantly higher (by 11.4 and 13.3%) than those of Pwm in soil with high Olsen-P. The microfloral contribution to yield was greater than that of soil P content in soil with high Olsen-P. Relative abundances of *Bacillus* and *Rhizobium* were enriched in the Pw treatment compared with the Pwm treatment. *Bacillus* showed a significant positive correlation with acid phosphatase (ACP) activity, and *Rhizobium* displayed significant positive correlations with ACP and ALP in soil with high Olsen-P, which may enhance P availability. Our findings suggested that the application of P fertilization only to wheat is practical in high P soils to ensure optimal production in the wheat and

Received 4 September, 2020 Accepted 14 December, 2020
YU Xiao-jing, E-mail: family19930428yxj@163.com; Correspondence LIU Zhi-guang, Tel: +86-538-8241531, E-mail: liuzhiguang8235126@126.com; ZHANG Min, Tel: +86-538-8241531, E-mail: minzhang-2002@163.com

doi: 10.1016/S2095-3119(20)63599-7

maize double cropping system and that the soil P availability and microbial community may collaborate to maintain optimal yield in a wheat–maize double cropping system.

Keywords: wheat–maize rotation, Olsen-P, alkaline phosphatase, phosphorus fertilization, calcareous soils

1. Introduction

Phosphorus (P) is an indispensable nutrient for growth and production of plants. Therefore, the application of P fertilizer is essential for high crop yields, particularly in low P soils (Li M *et al.* 2010; Han *et al.* 2014). The availability of P to plants in soils is limited, with P being either bound with calcium (Ca^{2+}) in calcareous soils or fixed by Fe and Al oxides in acidic soils (Zhang *et al.* 2001, 2014). In most production systems, the amount of P applied per unit area of crop land far exceeds the amount of P removal by the crop, which accounts for an annual P surplus of 13 kg of P ha^{-1} (Macdonald *et al.* 2011). Continuous and excessive application of P fertilizer annually results in increased P accumulation over the years, which contributes to low P use efficiency (PUE) of crop plants (Li W *et al.* 2010). Excessive P input into agricultural systems also contributes to environmental concerns such as eutrophication of water bodies. To mitigate these negative environmental impacts, there is a compelling need to develop and adapt to innovative P management technologies so that the P fertilizer rate across different crop production systems can be optimised and PUEs can be enhanced.

Optimal P management involves the application of suitable fertilizers at the appropriate time and rate, and by using an appropriate method (Da Cruz *et al.* 2017; Lu *et al.* 2019). Several researchers have focused on using alternative fertilizer types or novel additives, such as organic or coated P fertilizers, to improve fertilizer utilization (Da Cruz *et al.* 2017; Ahmad *et al.* 2018). A few reports regarding the farming patterns and fertilization methods with respect to PUE are also available. For example, in a rice–wheat rotation system, the application of P fertilizer only to wheat was recommended as the best P management practice to reduce P losses and enhance PUE for both crops in rotation (Wang *et al.* 2015, 2016a, b, 2018). The mechanisms responsible for maintaining adequate available soil P for both rice and wheat include: increased availability of P under anaerobic conditions in flooded soil during the rice season through the reduction of Fe^{3+} to Fe^{2+} (Wang *et al.* 2016b); release of P bound to Fe or Al in flooded soil due to chelation of Fe or Al by organic acids in rice rhizosphere (Wang *et al.* 2015); transformation of P into available forms by arbuscular mycorrhizal fungi (AMF) under the rice–wheat rotation.

Microorganisms play a vital role in controlling P availability, which can effectively mineralize soil organic P and transform insoluble P into soluble forms. Studies have shown that mycorrhizae such as *Claroideoglomus etunicatum* and *Acaulospora longula* usually provide the growth and nutritional benefits in soils with low available P (Oliveira *et al.* 2015). A proportion of nonrhizosphere soil available P could be absorbed by roots *via* AMF (Tisserant *et al.* 2013). When P availability in soil is low, AMF may cooperate with the microbial community in the hyphosphere to help transport nutrients from the nonrhizosphere soils with high P content or decomposed organic matter to host roots (Xu *et al.* 2018). Some inorganic phosphate-solubilizing bacteria (PSB) such as *Bacillus megaterium* M3 could efficiently transform immobilized P into bio-available P through high phosphatase activities (Turan *et al.* 2012; Khan *et al.* 2014). Reduction or elimination of P fertilizer application was found to promote the growth of Actinobacteria and Cyanobacteria, leading to increased alkaline phosphatase (*phoD*) gene abundance and stimulated hydrolysis of organic P (Wei *et al.* 2019). Phosphatases play a major role in facilitating the utilization of soil organic P by enhancing the hydrolysis and cleavage of PO, S–O, and PC-bonds of organic P (Pabis and Caroline 2016). A long-term field experiment with application of chemical and organic fertilizer under corn–wheat rotation showed that soil pH and labile P directly affect a specific microbial community composition and consequently influence the alkaline phosphatase (ALP) activity of soil (Luo *et al.* 2017). Therefore, the soil phosphatase activity and microbial composition can be improved by changing the soil management practices to optimize soil available P. However, whether this approach would be applicable for the wheat–maize double cropping system remains unclear.

We hypothesize that excess accumulation of P can be mitigated by applying P only to wheat under the wheat–maize double cropping system. The objective of this study was to evaluate and fine-tune the best management of P fertilization for the wheat–maize double cropping system. The specific objectives were to evaluate: (i) using soil Olsen-P levels as a basis for determining the P rate only for wheat to maximize crop yield and PUE; (ii) the relationship between soil Olsen-P and soil fungal and bacterial community composition; and (iii) the interaction of soil available P with the soil fungal and bacterial community that could maintain maize yield.

2. Materials and methods

2.1. Study site and materials

Experiments were performed during the years 2016–2018 in the New Fertilizer Experiment Station (36°20´N, 117°10´E) of Shandong Agricultural University, China. Soil samples were collected from a depth of 0–20 cm at a site with a 9-year history of wheat–maize double cropping (36°57´N, 117°58´E), located in Huantai County, Shandong Province, China. The soil type was classified as either Calcaric Ochri-Aquic Cambosol according to the Chinese Soil Taxonomy (CRGCST 2001) or Aquic Ustochrepts according to the USDA Soil Classification Taxonomy (SSS 2010). The soil samples (0–20 cm) represented high (H) and low (L) levels of Olsen-P. Table 1 presents the basic properties of the soil.

Soil samples were air-dried, sieved, and uniformly mixed. A total of 10 kg of soil was transferred to a polyvinyl chloride pot (29 cm in diameter, 22 cm in height). The experiment was conducted in a mesh enclosure to ensure ambient air temperature and humidity (determined by a recorder, S-THB-M008, U30-NRC, USA) throughout the experiment.

Conventional fertilizers included a fertilizer that used a controlled-release N to soluble N ratio of 7:3 (Zheng *et al.* 2017) by using resin-coated urea (43% N; 3 months of release period, Kingenta Ecological Engineering Group Co. Ltd., Shandong, China), uncoated urea (N, 46%), diammonium phosphate (N, 18%; P_2O_5, 46%), and potassium chloride (K_2O, 60%). A full dose of fertilizer was applied to each crop once at the time of planting.

2.2. Experimental design

A total of four treatments with P fertilization were included in four replications: (i) no P fertilization for wheat or maize (P0); (ii) conventional P fertilization for both crops, with a P_2O_5 rate of 150 kg ha^{-1} in wheat season and 75 kg ha^{-1} in maize season (Pwm); (iii) improved P fertilization only to wheat, with a P_2O_5 rate of 150 kg ha^{-1} in wheat season and 0 kg ha^{-1} in maize season (Pw); and (iv) P fertilization only to the wheat with the conventional annual amount, with a P_2O_5 rate of 225 kg ha^{-1} in wheat season and 0 kg ha^{-1} in maize season (Pw2). The treatment naming convention starts with H and L, representing the level (high or low) of soil Olsen-P. All the treatments included 450 kg ha^{-1} of N, with 225 kg ha^{-1} in both wheat and maize seasons and 225 kg ha^{-1} of K_2O with 75 kg ha^{-1} in wheat season and 150 kg ha^{-1} in maize season. Soil moisture, temperature, and rainfall were recorded at a meteorological station in the College of Agronomy, Shandong Agricultural University (Appendix A).

2.3. Plant and soil analysis

At the 12-leaf collar stage (V12) of maize in 2018, the following analyses were performed: (i) analysis of acid phosphatase (ACP) and ALP activity in the collected samples of fresh root and rhizosphere soil by using an ELISA Kit (Shanghai HengYuan Biological Technology Co., Ltd., Shanghai, China); (ii) analysis of ADP-glucose pyrophosphorylase (AGpase) of photosynthetic enzymes through enzyme-linked immunosorbent assay in the largest functional leaf samples collected; and (iii) determination of the net photosynthesis (P_n) rate of the largest functional leaf by using the LI-6400XT Portable Photosynthesis System (LI-Cor, Lincoln, NE, USA). After the harvest of each crop, the grain and straw were separated for the analysis of dry weight and total P (TP) content.

Furthermore, at the maize seedling stage in 2018, V12 and the milk ripening stage *in-situ* soil solution were sampled at a 15-cm depth by using a MiniRhizon sampler (SMS, AgriEco, China) one day after the pot was irrigated. Electrical conductivity (EC) and pH were measured using a conductivity meter and a pH meter, respectively. Concentrations of water-soluble P and Ca^{2+} were determined using ICP-MS (Model IRISER/S, USA). After harvesting the crops, all the treated soil samples (0–20 cm in depth) were collected. Samples were air-dried, ground and sieved (<2-mm sieve), and stored at room temperature. Soil pH, Olsen-P, TP, NO_3^--N, NH_4^+-N, TN, and soil organic matter (SOM) were measured according to the methods described previously by Lu (2000).

2.4. Soil DNA extraction and Illumina MiSeq sequencing

Soil DNA was extracted from 0.5 g soil by using the Power Lyzer Power Soil DNA Isolation Kit (Omega Bio-Tek D5625, USA). An ultraviolet (UV) spectrophotometer (Thermo NanoDrop2000, USA) was used to determine the quality and concentration of the extracted DNA.

Universal primer pairs 515F (5´-GTGCCAGCMGCCGC

Table 1 Properties of high (H) and low (L) Olsen-P soils

Sample	pH	Olsen-P (mg kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	SOM (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
H	8.01	30.36	1.27	1.21	15.58	11.46	4.72
L	8.20	9.78	1.23	0.75	16.29	11.97	4.49

GG-3′) (Chen *et al.* 2018) and 907R (5′-CCGTCAATTC MTTTRAGTTT-3′) were used to expand the V4–V5 region of the bacterial 16S ribosomal RNA gene. The primers used for fungi were ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) (Li *et al.* 2018). PCR reactions were performed in a thermocycler under the following conditions: 95°C for 3 min, 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 10 min (ABI GeneAmp 9700, USA). Amplicons were quantified using QuantiFluor™-ST (Promega, USA) in accordance with standard protocols and sequenced on the Illumina MiSeq PE300 Platform. Data were processed using the Quantitative Microbiological Ecology Analysis (QIIME) pipeline (version 1.17), with raw sequences of >200 bp, average quality score of >20, and no ambiguous base calls.

Operational taxonomic units (OTUs) were clustered with 97% similarity for the statistical analysis of bacterial and fungal biological information. OTU representative sequences were aligned with the UNITE Database and Greengenes Database for fungi and bacteria, respectively. The rarefied OTU profiles were generated at depths of 31 320 sequences per sample for fungi and at depths of 18 743 sequences per sample for bacteria. The sequences were deposited in the NCBI Sequence Read Archive (BioProject ID: PRJNA597955).

2.5. Statistical analysis

Statistical Analysis System (SAS; ver. 9.2) was used for performing ANOVA and Duncan's multiple range test (P<0.05 to accept; SAS, 2010). Alpha diversity metrics were calculated using Mothur. Principal co-ordinate analysis (PCoA) was based on the unweighted UniFrac distance. Adonis analyses were performed using the unweighted UniFrac distance matrices to determine the significance of microbial community differences through the 'vegan' package in R. Pearson correlation was used to determine the relationships between yield, soil properties, and bacterial/fungal community composition by using the cor function of the base R package 'stats', and the correlation results were visualized using the corrplot.mixed function of the R package 'corrplot'. Structural equation modelling (SEM) was applied to explore the interrelationships between soil P characteristics, bacterial and fungal communities, and yield. The vegan packaging in R was used for SEM analysis (Zhao *et al.* 2019). The relationship between the microbial taxa and phosphatase activity was estimated on the basis of Spearman correlation. Coefficients and statistically significant correlations (P<0.05) were selected to construct a correlation network. Cytoscape ver. 3.6.0. was applied to visualize the correlation networks.

The PUE was calculated using the formula (Devkota *et al.* 2013):

PUE (%)=(Cumulative P absorbed from plants with P treatment–Cumulative P absorbed from plants without P treatment)/Total application of P fertilizer with P treatment×100

3. Results

3.1. Crop yield and physiological characteristics

The fertilization treatments evaluated in this study were found to significantly affect the crop yield under different soil Olsen-P levels (Table 2). At the end of the 2-year study period, maize yields were similar across both Pw and Pwm treatments in high Olsen-P soil (Table 2). However, in low Olsen-P soil, the maize yield in Pw treatment was found to be reduced by 22.44 and 10.11% compared with that in Pwm treatment during 2017 and 2018, respectively (P<0.05). When the P fertilizer rate of Pw treatment was increased to that of Pw2 treatment, a stable maize yield of Pw2 treatment comparable with that of Pwm treatment was maintained. Combined yields of wheat and maize in the Pw2 (HPw2 and LPw2) treatment were not found to vary significantly from those of the Pwm (HPwm and LPwm) treatment. These results suggested that the yield variation in the Pw treatment is caused by the amount of P fertilizer supplied.

Total yields in the LP0 and HP0 treatments were reduced by 61.96–78.59% and 8.66–13.48%, respectively, compared with those in the conventional P treatments (LPwm and HPwm) during 2016–2018. These results indicated that the soil Olsen-P level is an important indicator for potential crop yields. Wheat yield was not found to differ significantly (P>0.05) between the Pw2 and Pwm treatments, yet the rate of applied P in the Pw2 treatment was approximately 50% greater than that in the Pwm treatment. Therefore, excessive P application does not improve the yield. The results indicate that the annual P input can be reduced by 33.3% without negative effects on the yield in high P soil.

The daily average temperature of wheat yield in 2018 was sharply increased from −0.2°C to 3.6°C more than the normal temperature (Appendix A). Different P fertilization treatments also affected the photosynthesis rates of crops, which might partially explain the difference in yield between the treatments (Appendix B). Compared with Pwm (HPwm and LPwm) treatment, the net photosynthetic rate and AGPase activity were not found to decrease significantly for the Pw (HPw and LPw) treatments. The AGPase activity ranged from 1.58 to 2.00 U g^{-1} for high Olsen-P soil and from 1.32 to 1.82 U g^{-1} for low Olsen-P soil.

3.2. Soil P and nutrient status

Fertilization treatments significantly affected the soil Olsen-P and TP contents (Fig. 1). At the end of the 2-year study period, in high Olsen-P soil, the soil Olsen-P content decreased by 27.4% and increased by 45.8%, as compared with the initial level (30.36 mg kg^{-1}), after the Pw and Pwm treatments, respectively. Similarly, the TP content remained unchanged and increased by 20.6% compared with the initial soil TP content (1.21 g kg^{-1}), following the HPw and HPwm treatments, respectively. Therefore, P application in high Olsen-P soil exceeded the P removal by the crops after the Pwm treatment, whereas the P level in soil after Pw treatment was adequate to meet the crop yield for at least two years without excessive residual P accumulation in the soil. The PUE in Pw treatment increased by 10.8 and 6.3%, compared with Pwm treatment in high and low Olsen-P soils, respectively (Appendix C; $P>0.05$). The annual average PUE of high Olsen-P soil (12.35–18.27%) was lower than that of low Olsen-P soil (25.87–29.05%) (Appendix C).

At the V12 stage in high P soil, the soil solution pH value decreased by 0.19, 0.21, and 0.18 with the P0, Pw, and Pw2 treatments, respectively, compared with that after the Pwm treatment (Table 3; $P>0.05$). The corresponding values in low P soil decreased by 0.18, 0.12 and 0.06 (Table 3; $P>0.05$). The soil solution pH values after Pw treatment at the seedling stage and milk stage were 0.63 and 0.50 units lower, respectively, than those observed after Pwm treatment in low Olsen-P soil (Table 3; $P<0.05$).

At the seedling stage, the soil water-soluble P concentration after Pw treatment (0.61 mg L^{-1}) was 77.8% greater than that after P0 treatment (0.34 mg L^{-1}) in high Olsen-P soil, whereas the soil water-soluble P concentration after LPw treatment (0.45 mg L^{-1}) was similar to that after LP0 treatment (0.44 mg L^{-1}). At the V12 stage, EC and water-soluble P values of Pw (HPw or LPw) treatment soil solutions were similar after the Pw and Pwm treatments regardless of whether in high or low P soils. The Ca^{2+} concentration of the soil solution after Pw treatment at the V12 stage was 48.8% greater than that at the seedling stage.

3.3. Phosphatase activity in soil and root

The ACP and ALP activities in soil and root were significantly influenced by the fertilization treatments (Table 4). No significant differences were observed between the HPw and HPwm treatments (Table 4; $P>0.05$). However, the ACP activity in roots after HPw treatment was 8.7% lower than that after the HPwm treatment. This result suggests that plant roots as well as soil microbial secretion contribute to the ACP activity in soil with high Olsen-P. In low Olsen-P soil, the soil ACP activity after Pw treatment was significantly lower (8.6%) than that after Pwm treatment ($P<0.05$).

3.4. Soil microbial community composition

Ascomycota, Basidiomycota, and Zygomycota were found to be the dominant fungi, whereas Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Planctomycetes, and Firmicutes were found to be the dominant bacteria in the soil across all the treatments (Appendix D). In high Olsen-P soil, most of the bacterial counts were not found to vary significantly between the Pw and Pwm treatments, except for the Proteobacteria count, which was 11.4% greater after the Pw treatment compared with the Pwm treatment ($P<0.05$). The relative abundance of Firmicutes after the Pw2 treatment was found to be 49.2% greater than that after the Pwm treatment ($P<0.05$). In low Olsen-P soil, the relative abundance of Proteobacteria after Pw2 treatment was found to be 16.4% greater than that after the Pwm treatment (Appendix E; $P<0.05$). The relative abundance of Chloroflexi in the Pw and Pw2 treatments was found to

Table 2 Wheat and maize yield responses to four P fertilization treatments in high and low Olsen-P soils

Year	Treatment[1)]	Soil with high Olsen-P				Soil with low Olsen-P			
		Wheat yield (g/pot)	Maize yield (g/pot)	Total yield (g/pot)	Change from Pwm (%)	Wheat yield (g/pot)	Maize yield (g/pot)	Total yield (g/pot)	Change from Pwm (%)
2016/2017	P0	73.27 b	77.40 c	150.67 b	–13.48	17.87 b	49.75 c	67.62 c	–61.96
	Pwm	90.15 a	84.02 b	174.16 a	–	91.55 a	86.23 a	177.77 a	–
	Pw	87.17 a	88.71 ab	175.88 a	0.99	94.45 a	66.88 b	161.32 b	–9.25
	Pw2	88.86 a	90.19 a	179.05 a	2.81	95.28 a	83.96 a	179.23 a	0.82
2017/2018	P0	35.23 a	115.47 b	150.70 b	–8.66	16.33 b	19.57 c	35.90 c	–78.59
	Pwm	34.63 a	130.35 a	164.98 a	–	33.43 a	134.27 a	167.70 a	–
	Pw	37.68 a	125.85 a	163.53 a	–0.88	32.73 a	120.70 b	153.43 b	–8.51
	Pw2	38.73 a	131.71 a	170.44 a	3.31	32.57 a	124.50 ab	157.07 ab	–6.34

[1)] P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount.

Means followed by the same letter in each column and for each year are not significantly different based on one-way ANOVA followed by Duncan's multiple-range tests ($P>0.05$). – indicates no data.

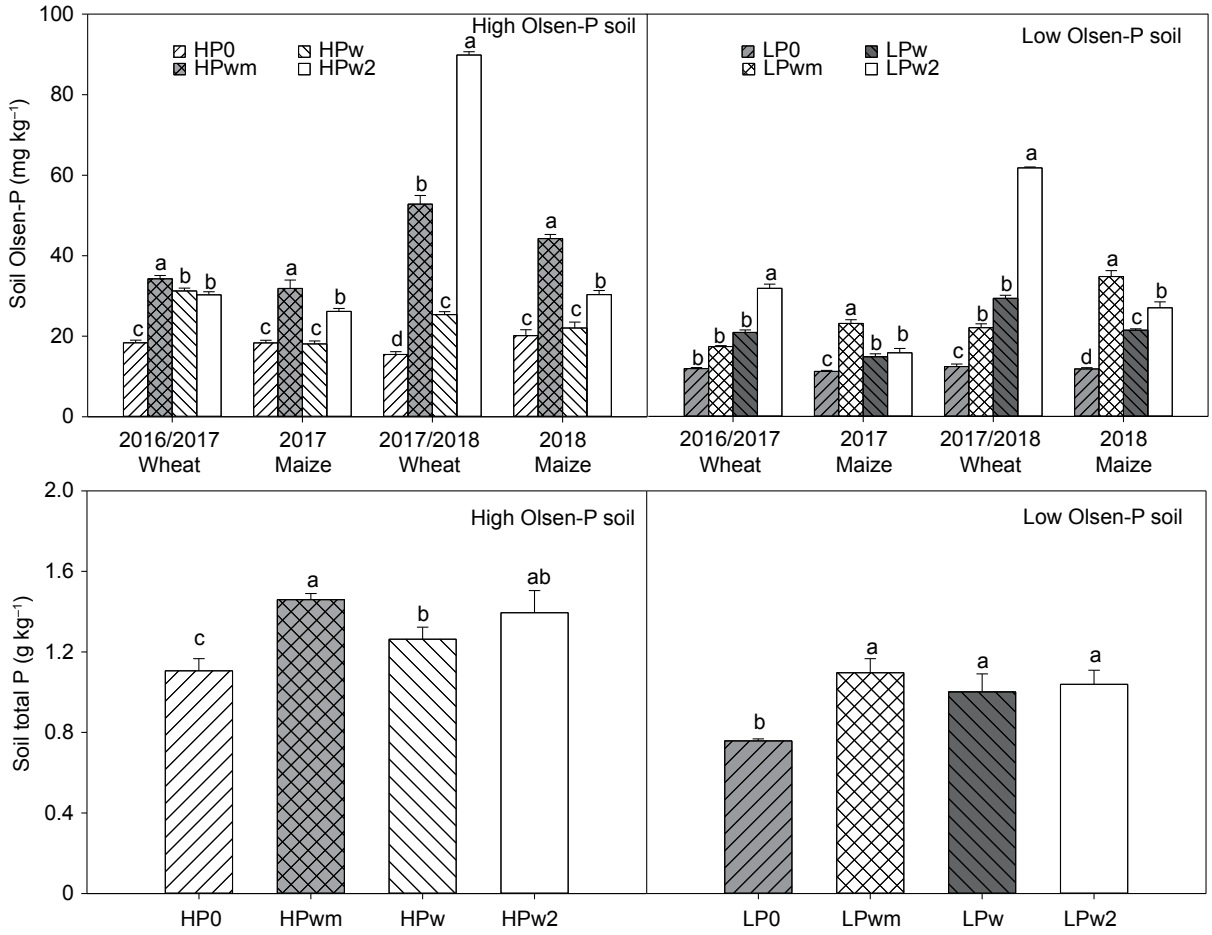


Fig. 1 Soil Olsen-P and total phosphorus (P) contents of four phosphate fertilization treatments in a wheat and maize rotation at wheat/maize harvest time of four seasons, and total P at maize harvest time in 2018. P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount. Error bars represent SD of three replicates. Within each graph, means followed by the same letter are not significantly different based on a one-way ANOVA followed by Duncan's multiple-range test (P>0.05) in the same soil.

Table 3 *In-situ* soil solution pH, electrical conductivity (EC) and concentrations of water-soluble P and Ca^{2+} during three critical periods of four different phosphate fertilization treatments during the maize growing season of 2018

Maize growth stage	Treatment[1)]	Soil with high Olsen-P				Soil with low Olsen-P			
		pH	EC (ms cm^{-1})	Water-soluble P (mg L^{-1})	Ca^{2+} (mg L^{-1})	pH	EC (ms cm^{-1})	Water-soluble P (mg L^{-1})	Ca^{2+} (mg L^{-1})
Seedling	P0	7.93 a	6.88 ab	0.34 d	742.2 a	8.01 a	4.99 b	0.44 b	661.10 ab
	Pwm	7.93 a	7.87 a	0.82 b	770.0 a	8.03 a	4.94 b	0.87 a	452.40 c
	Pw	7.95 a	2.52 c	0.61 c	278.5 c	7.40 b	6.60 ab	0.45 b	575.80 bc
	Pw2	8.11 a	3.96 bc	1.22 a	368.7 b	8.05 a	6.94 a	0.89 a	712.50 a
12-leaf collar	P0	7.78 a	4.77 b	0.63 a	475.8 ab	7.52 a	4.04 c	0.63 a	437.10 b
	Pwm	7.97 a	4.30 b	0.62 a	478.8 ab	7.70 a	5.80 b	0.63 a	539.00 a
	Pw	7.76 a	4.18 b	0.57 a	414.3 b	7.58 a	6.99 a	0.59 a	592.50 a
	Pw2	7.79 a	6.02 a	0.64 a	542.7 a	7.64 a	5.01 b	0.62 a	546.40 a
Milky ripe	P0	7.83 a	4.24 a	0.68 bc	92.9 b	7.58 b	0.52 b	0.84 a	70.24 b
	Pwm	7.83 a	0.78 c	0.90 a	129.5 b	8.13 a	0.84 a	0.77 a	100.18 a
	Pw	7.87 a	2.29 b	0.55 c	272.5 a	7.63 b	0.78 a	0.81 a	115.94 a
	Pw2	7.83 a	0.78 c	0.74 ab	133.2 b	7.82 ab	0.47 b	0.88 a	56.95 b

[1)] P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount.

Means in each column and for each growth stage followed by the same letter are not significantly different at P=0.05, based on ANOVA followed by Duncan's multiple range test.

Table 4 Acid phosphatase and alkaline phosphatase enzyme activities in the soils and roots of four different P fertilization treatments at the 12-leaf collar (V12) stage during the maize season in 2018[1)]

Treatment	Soil acid phosphatase (U g^{-1})		Soil alkaline phosphatase (U g^{-1})		Root acid phosphatase (U g^{-1})		Root alkaline phosphatase (U g^{-1})	
	H-P	L-P	H-P	L-P	H-P	L-P	H-P	L-P
P0	0.648 a	0.513 b	0.095 a	0.097 b	0.706 b	0.764 a	0.117 b	0.111 b
Pwm	0.619 a	0.569 a	0.083 b	0.095 b	0.773 a	0.843 a	0.139 a	0.134 b
Pw	0.686 a	0.520 b	0.094 a	0.125 a	0.706 b	0.771 a	0.157 a	0.163 a
Pw2	0.625 a	0.604 a	0.081 b	0.095 b	0.718 b	0.808 a	0.137 ab	0.119 b

[1)] H-P, high Olsen-P soil; L-P, low Olsen-P soil. P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount.
Means in each column followed by same lowercase letters are not significantly different at P=0.05, based on ANOVA followed by Duncan's multiple range test.

be 20.5–16.1% lower than in the Pwm treatment (Appendix E; P<0.05). The effects of reducing P fertilizer input on the relative abundances of most genera are shown in Fig. 2. Relative abundances (>0.1%) of *Bacillus*, *Rhizobium*, *Blastopirellula*, and *Nitrolancea* bacteria were significantly greater after the Pw treatment than after the Pwm treatment in high Olsen-P soil (Fig. 2-D).

PCoA using unweighted UniFrac was used to further investigate the differences in microbial communities across different P fertilizer treatments (Fig. 3). The results showed that P fertilization significantly affects the composition of the fungal community in both high Olsen-P (HP-fungi) (Fig. 3-B; Adonis, R^2=0.349, P=0.002) and low Olsen-P soils (LP-fungi) (Fig. 3-C; Adonis, R^2=0.339, P=0.012). Furthermore, the impacts on bacterial communities were significant in soil with low Olsen-P (LP-bacteria) (Fig. 3-F; Adonis, R^2=0.322, P=0.001) and marginal in soil with high Olsen-P (HP-bacteria) (Fig. 3-E; Adonis, R^2=0.291, P=0.055). The community composition of bacteria and fungi after P0 treatment was clearly different from that after other P application treatments (Pwm, Pw, and Pw2) in both high and low Olsen-P soils (Fig. 3-B, C, E, and F). Microbial communities of soils in the HPwm and HPw2 treatments were generally similar with overlap in the plot. The fungi and bacteria showed a trend of P0→Pw→Pw2/Pwm, consistent with the trend observed for Olsen-P and TP contents (Fig. 1).

3.5. Relationships between the soil fungal and bacterial community composition and soil properties

Pearson correlation analysis was used to determine the relationships between soil properties, microbial characteristics, and yield (Fig. 4). The PC1 and PC2 characteristic values (HP_Bac_PC1/PC2, HP_Fun_PC1/PC2, LP_Bac_PC1/PC2, LP_Fun_PC1/PC2) of the obtained PCoA results (Fig. 3-B, C, E, and F) were selected to characterize the main characteristics of the soil fungi and bacteria community (Feng *et al.* 2020). The results showed that Olsen-P and TP contents are positively correlated with crop yield (Fig. 4). The HP_Fun_PC1 and HP_Bac_PC1 were significantly correlated with crop yield, Olsen-P, TP, and ATP activity in soil with high Olsen-P (Fig. 4-A). The LP_Fun_PC2 and the LP_Bac_PC1 were significantly correlated with crop yield, Olsen-P, and TP content in soil with low Olsen-P (Fig. 4-B). These results further supported the fact that P fertilization significantly affects the soil microbial community composition. Furthermore, the ALP activity of high Olsen-P rhizosphere soil was found to be significantly and negatively correlated with the Olsen-P and TP contents and yield, and its significant correlations with HP_Bac_PC1 and HP_Fun_PC1/2 were also observed. However, the ALP activity of low Olsen-P rhizosphere soil was found to have significant positive correlations with EC and LP_Bac_PC2. The ACP activity of high Olsen-P rhizosphere soil was found to have a significant positive correlation with Bac_Shannon and a significant negative correlation with Fun_Shannon. Additionally, the ACP activity in low Olsen-P rhizosphere soil was found to be significantly and positively correlated with LP_Bac_PC1 and Olsen-P.

To further characterize the potential relationships between soil microflora, soil P characteristics, and yield, we constructed SEM models (Fig. 5). As shown in Figs. 3 and 4, HP_Bac_PC1 and HP_Fun_PC1 in soil with high Olsen-P and LP_Bac_PC1 and LP_Fun_PC2 in soil with low Olsen-P were selected to further represent the bacterial and fungal community. The results suggested a significant interaction between soil P and microflora characteristics (Fig. 5-A and C). The combination of soil P and microflora significantly explained the variation in yield by 58 and 98% in soil with high and low Olsen-P, respectively (Fig. 5-B and D). In soil with high Olsen-P, the contribution of microflora to yield was greater than that of soil P, whereas in soil with low Olsen-P, the contribution of soil P to yield was greater than that of microflora (Fig. 5-A and C). This result indicated that soil P is the main limiting factor for yield in soil with low Olsen-P, while the contribution of soil microflora to yield is greater than that of soil P level in soil with high Olsen-P.

Spearman analysis was used to explore the correlations

between soil phosphatase and microbial taxa for selective biomarker microbes (Fig. 6). In soil with high Olsen-P, *Bacillus* displayed a significant positive correlation with the ACP activity, *Rhizobium* displayed significant positive correlations with both ACP and ALP activities, and *Blastopirellula* and *Nitrolancea* displayed significant positive correlations with the ALP activity. In soil with low Olsen-P, *Rhizobium* displayed a significant positive correlation only with the ACP activity.

4. Discussion

4.1. Feasibility of the reduced phosphate fertilization scheme

The critical soil P level determines the effectiveness and utilization of P fertilizer in increasing the crop yield (Khan *et al.* 2018). Our results indicated a reduction in the total yield without the application of P fertilizer by 8.66–13.48%

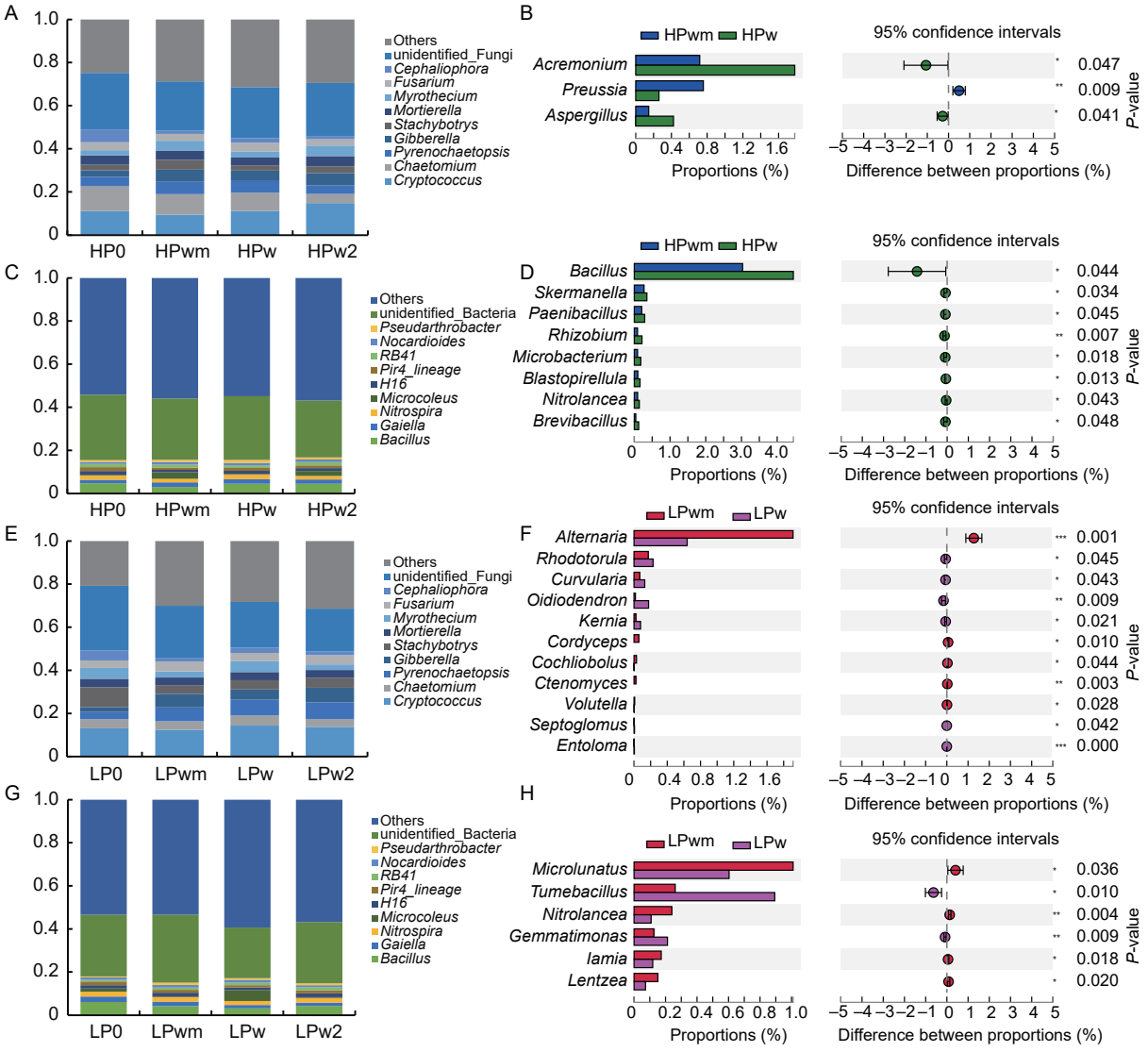


Fig. 2 Relative abundances of the top 10 dominant soil fungi and bacterial communities at the genus level and fungal and bacterial genera with significant differences in their relative abundances (relative abundances>0.1%) between Pw and Pwm treatments at the 12-leaf collar (V12) stage of the maize season in 2018. A and E, top 10 dominant fungal genera. C and G, top 10 dominant bacterial genera. B and F, the fungal genera with significant differences of their relative abundances (relative abundances>0.1%) between Pw and Pwm treatments based on Student's *t*-test, respectively (P<0.05; n=3). D and H, the bacterial genera with significant differences of their relative abundances (relative abundances>0.1%) between Pw and Pwm treatments based on Student's *t*-test, respectively (P<0.05; n=3). P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount. Error bars represent SD of three replicates. *, P<0.05; **, P<0.01; ***, P<0.001.

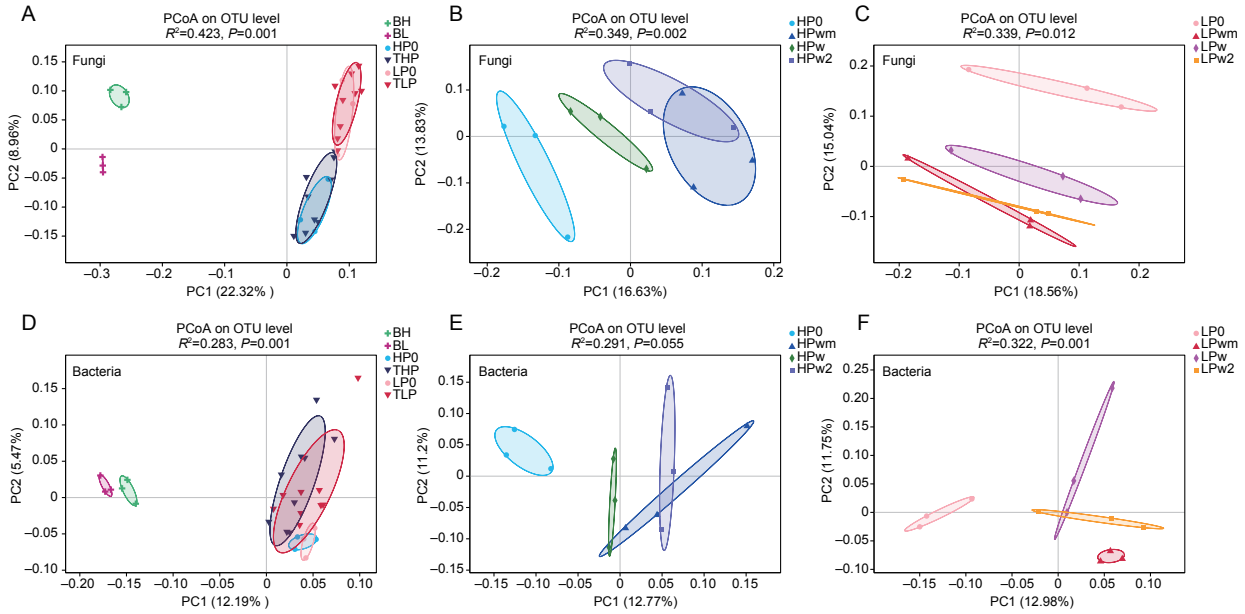


Fig. 3 Fungal and bacterial community composition dissimilarity across the four phosphate fertilization treatments and original soils visualized using the principal coordinates analysis (PCoA) based on the unweighted-UniFrac. A and D, original soils and four P fertilization treatments. B and E, four P fertilization treatments in high Olsen-P soil. C and F, four P fertilization treatments in low Olsen-P soil. P0, no P fertilization for wheat or maize; Pwm, conventional P fertilization for both crops; Pw, improved P fertilization only to wheat; Pw2, P fertilization only to the wheat with the conventional annual amount; BH, original soil of high Olsen-P; BL, original soil of low Olsen-P; THP, HPwm, HPw and HPw2; TLP, LPwm, LPw and LPw2.

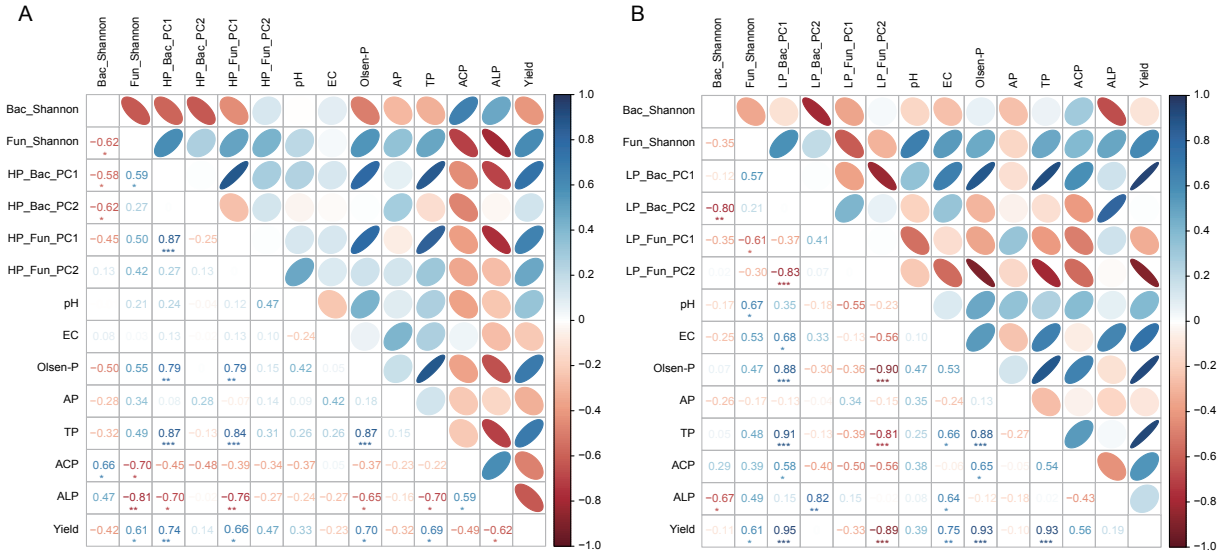


Fig. 4 Correlation analysis of the relationships between the yield, soil properties, and fungal/bacterial community composition under different Olsen-P levels. A, high Olsen-P soil. B, low Olsen-P soil. Bac_Shannon, the bacterial community diversity; Fun_Shannon, the fungal community diversity; HP_Bac_PC1/PC2, soil bacterial communities in high Olsen-P soil; HP_Fun_PC1/PC2, soil fungal communities in high Olsen-P soil; LP_Bac_PC1/PC2, soil bacterial communities in low Olsen-P soil; LP_Fun_PC1/PC2, soil fungal communities in low Olsen-P soil; EC, electrical conductivity; AP, water-soluble P; TP, total P; ACP, soil acid phosphatase activity; ALP, soil alkaline phosphatase activity. Red indicates negative correlation and blue indicates positive correlation. The darker the color, the stronger the correlation, and *vice versa*. Ellipse roundness indicates the degree of correlation, i.e., the greater the roundness, the stronger the correlation. The number in box indicates the correlation coefficient. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

in the high Olsen-P soil (30.36 mg kg^{-1}) and by more than 60% in the low Olsen-P soil (9.78 mg kg^{-1}). Meanwhile, the annual average PUE of high Olsen-P soil (12.35–18.27%) was found to be lower than that of low Olsen-P soil (25.87–

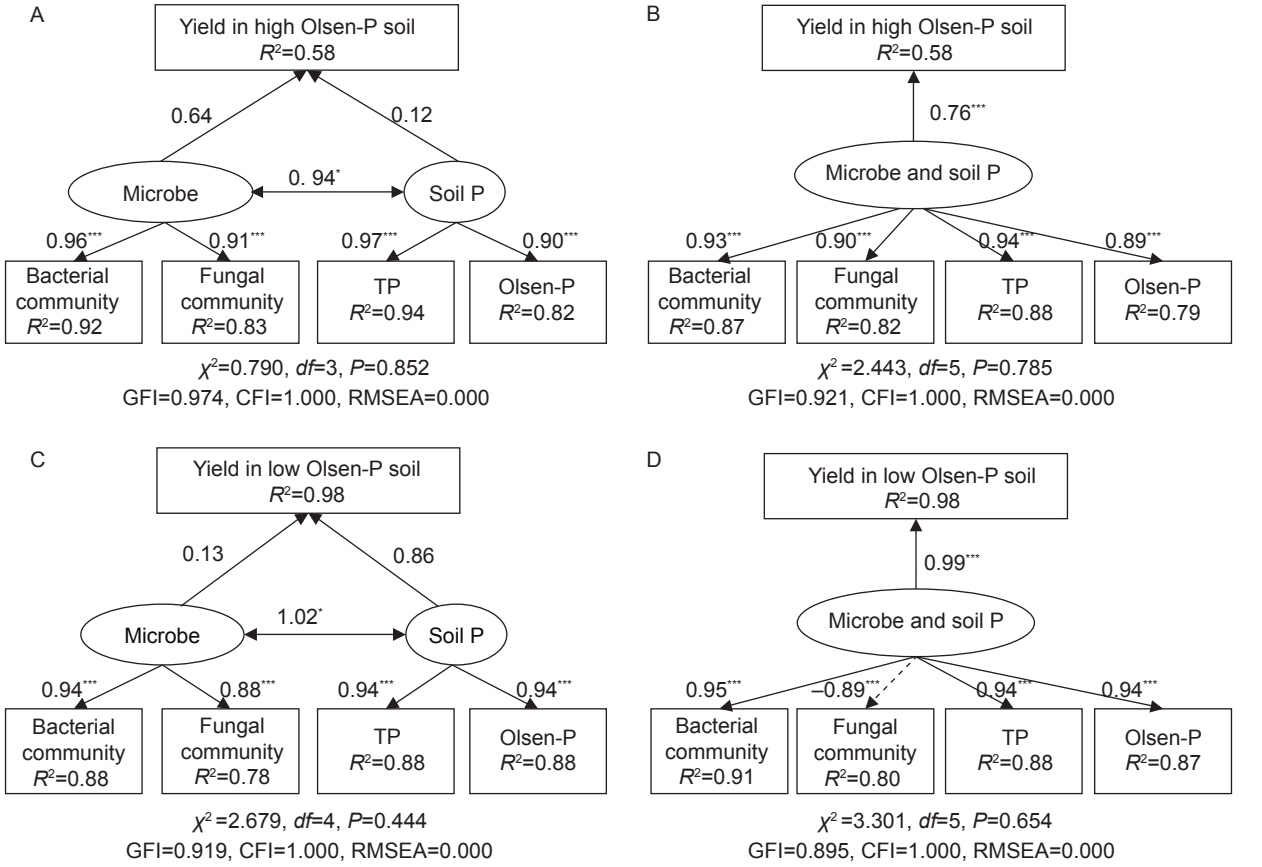


Fig. 5 Structural equation model of the potential relationship among maize yield, soil P, and microflora characteristics in different Olsen-P levels. A and B, high Olsen-P soil. C and D, low Olsen-P soil. The main characteristics of the bacterial community and fungal community in A and B were represented by HP_Bac_PC1 and HP_Fun_PC1 in high Olsen-P soil, respectively, and those in C and D were represented by LP_Bac_PC1 and LP_Fun_PC2 in low Olsen-P soil. GFI, goodness-of-fit index; CFI, comparative fit index; RMSEA, root-mean-square errors of approximation. Adjacent numbers in the same direction as the arrow are path coefficients, and the width of the arrow is proportional to the size of the path coefficients. The R^2 value indicates the proportion of variance explained for each variable. *, P<0.05; **, P<0.01; ***, P<0.001.

29.05%) (Appendix C). These results suggest that the P content is an important factor that limits the crop yield and PUE. The soil Olsen-P content of 20 mg kg^{-1} is an ecological threshold that could meet the demand of high crop yields (Li *et al.* 2011), which is consistent with the present study, wherein the Olsen-P content of the HPw treatment remained higher than 20 mg kg^{-1} over two years of rotation, ensuring a stable maize yield. However, the initial Olsen-P content in low Olsen-P soil was only 9.78 mg kg^{-1}, which resulted in a decrease in maize yield by 10.11–22.44% after the LPw treatment compared with the LPwm treatment (Tables 1 and 2). These results support our hypothesis that the application of P only in the wheat season is an appropriate management scheme and is determined by the soil Olsen-P content. Moreover, the HPw treatment increased the PUE by 10.8% as compared with the HPwm treatment (P>0.05, Appendix C). Similar fertilization reduction schemes were used in a 4-year rice–wheat rotation system that increased the PUE by 1.2–3.6% (P>0.05) (Wang *et al.* 2016b). Meanwhile, the TP content after the HPwm treatment increased by 20.6% compared with that of the base soil (1.21 g kg^{-1}). In contrast, the TP content after the HPw treatment remained steady. In general, a reasonable reduction in the amount of P fertilizer will reduce the accumulation and loss of P and increase the PUE while ensuring optimum crop yields.

The maize growth environment may maintain yield under the condition of reduced P fertilizer application. Firstly, the wheat–maize rotation system featured a low soil temperature environment during the wheat season (average soil temperature of 8.32°C) and a high soil temperature environment during the maize season (as high as 26.33°C, Appendix A). Seasonal low temperatures reduce the soil microbial activity and nutrient turnover rate (Suseela *et al.* 2012), whereas the soil temperature ranging from 10 to 25°C doubles the mineralization rate (Macdonald *et al.* 1995). The average temperature during the maize

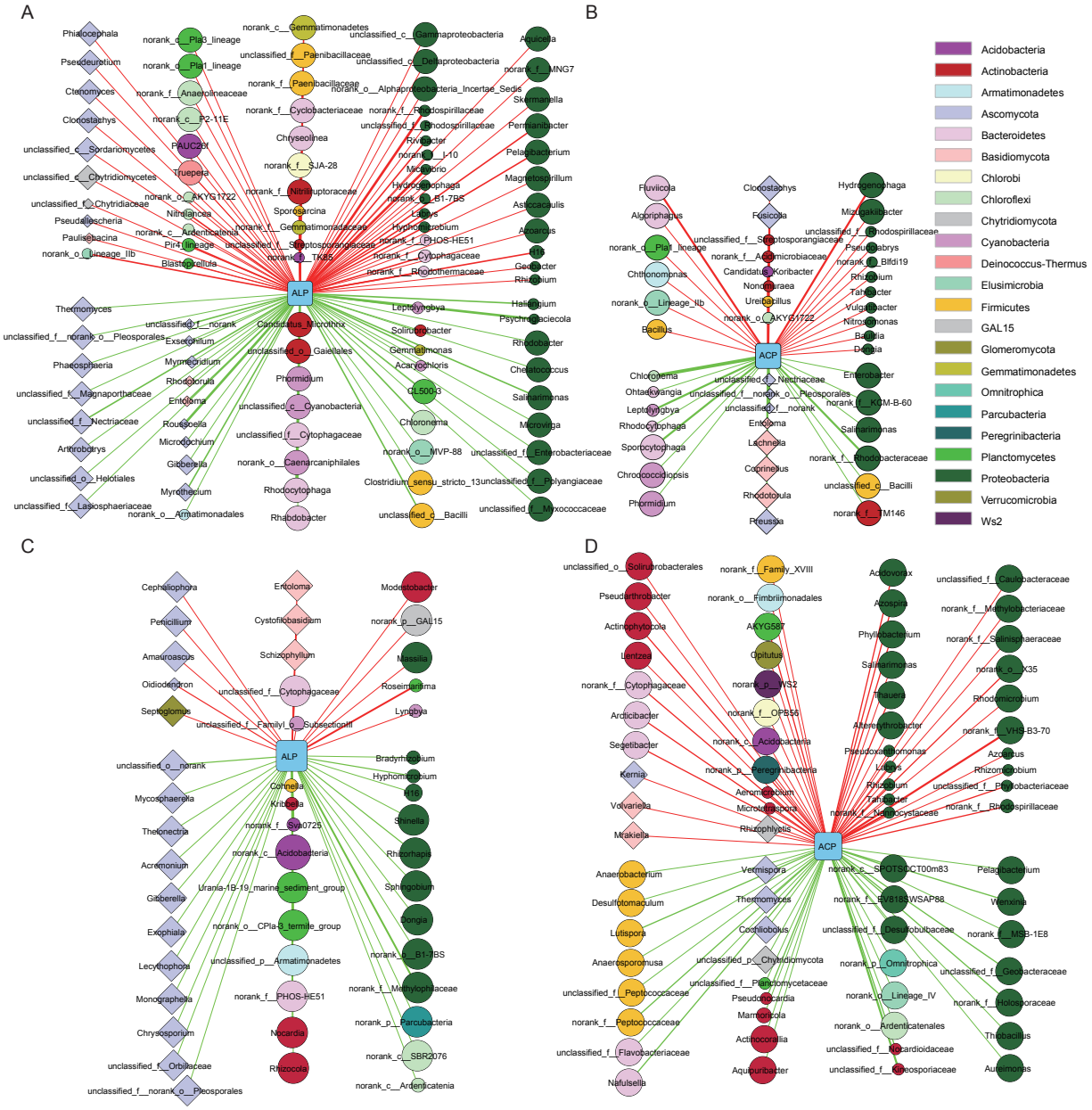


Fig. 6 Correlation network between phosphatase and microorganisms under different Olsen-P levels. A and B, high Olsen-P soil. C and D, low Olsen-P soil. ACP, soil acid phosphatase activity; ALP, soil alkaline phosphatase activity. The square in the middle represents the key environmental factors, while the outer circle is bacteria, and the rhombus is fungi, which are significantly related genera of microorganisms. The shape size represents the relative abundance level and the color of the shape indicates different phylum levels. The color of each line indicates correlation, green indicates negative correlation and red indicates positive correlation. The line thickness represents the significance level, with thin lines representing $0.01<P<0.05$ and thick lines representing $P<0.01$.

season is 18.01°C higher than that during the wheat season, which may increase the activity of enzymes, such as phytase and glycerol phosphatase, thereby enhancing the mineralization of organic P (Arenberg and Arai 2018; Andry *et al.* 2019). Secondly, diffusion is an important mechanism for the absorption of soil P by maize roots (Jia *et al.* 2018). Variations in the soil moisture content from 0.3 to 0.2 cm^3 cm^{-3} decreases the P diffusion rate by 98% (Sun *et al.* 2017). In the present study, the soil moisture content during maize season (0.32 cm^3 cm^{-3}) was 1.68 times higher than that during wheat season (0.19 cm^3 cm^{-3}), which was conducive to enhancing P transport through root uptake (Appendix A). Thirdly, the dry–wet alternation can enhance aggregate turnover to increase the organic matter decomposition rate (Denef *et al.* 2014). High amounts of rainfall and irrigation during the maize season resulted in

frequent dry–wet alternations (Appendix A). The conversion of microbial biomass P during dry–wet alternation leads to enhanced water-soluble P (Turner and Haygarth 2001; Song *et al.* 2018). Under the flooded condition, O_2 is deficient, and the Eh value is gradually reduced. The elements Mn and Fe are sequentially reduced by electrons, thereby releasing P nutrients (Jenkinson and Franzmeier 2006). Long-term experiments have shown that winter wheat responds to P fertilizer better than summer maize (Yuan *et al.* 2017) and that maize utilizes P more effectively than wheat (Tang *et al.* 2009). In general, these processes may help to maintain a stable soil water-soluble P level for maize uptake (Table 3). We further discuss the interaction between soil available P and microbial communities.

Therefore, P application in Pwm treatment exceeded the P removal by the crops in high Olsen-P soil, whereas in Pw treatment the soil P level was adequately maintained to meet the crop yield for at least two years without excessive residual P accumulation in the soil. The management of P application aims to optimize the PUE to ensure yield, which can maintain the soil Olsen-P content within limits by avoiding either depletion or surplus accumulation. The soil Olsen-P content of 20 mg kg^{-1} could be used to evaluate the effectiveness and utility of the reduced P fertilization scheme by applying P fertilizer only to wheat. In the North China Plain, the wheat–maize double cropping system occupies an area of nearly 14 million ha, with the Olsen-P content mostly higher than 20 mg kg^{-1} (Li *et al.* 2011; MOA 2013; Xue *et al.* 2015). The implementation of the P reduction fertilization scheme proposed in this study would save 1.05 million tons of P fertilizer, and considering the current price of diammonium phosphate (378.0 USD t^{-1}, 48% P_2O_5), the benefit would translate to a saving of 826.88 million USD per year.

4.2. Effects of soil phosphorus availability on the microbial community composition and soil phosphatase activity

Fertilizer application and soil P bioavailability greatly influence the soil microbial community composition (Li *et al.* 2015; Xia *et al.* 2019). Our results showed that the P fertilization scheme significantly affects the composition of both bacterial and fungal communities in soil with a low Olsen-P content (Fig. 3-C and F); however, the composition of fungal communities in soil with high Olsen-P was affected by the proposed fertilization scheme (Fig. 3-B). Islam *et al.* (2011) observed that chemical fertilizers increase the microbial biomass and activity but do not significantly change the bacterial community structure. The microbial communities after HPwm and HPw2 treatments were similar, which is most likely due to the same amount of applied fertilizer and soil TP content. SEM results indicated that the soil Olsen-P content limits the yield in soil with low Olsen-P, whereas the microflora contributes to yield in soil with high Olsen-P.

Soil microorganisms drive P cycling through excretion of extracellular enzymes such as ALP (*phoD*) and ACP (encoded by *olpA*), which mineralizes organic P (Sharpley 1985; Liang *et al.* 2020). Studies have shown that bacteria are the main source of phosphatase, especially ALP, and our correlation analysis further indicated that the ALP activity is significantly correlated with the Olsen-P content and bacterial community characteristics in soil with high Olsen-P (Fig. 4). Unlike ALP, the ACP activity is mainly derived from plants, and the enzyme is also secreted by bacteria and fungi (Chen *et al.* 2019). The present study indicated that the ACP activity is significantly and positively correlated with Bac_Shannon in soil with high Olsen-P (Fig. 4) and suggested that the reduced P fertilization scheme might promote the secretion of ACP by bacteria.

An increase in the abundance or diversity of bacteria leads to an increase in the phosphatase activity (Zhang *et al.* 2020). Our results showed that soils under the HPw treatment display 11.4% more Proteobacteria and 13.3% higher ALP activities than soils under the HPwm treatment (Table 4; Appendix E). Firmicutes and Proteobacteria contain the *phoD* gene, which is the most common ALP gene (Ragot *et al.* 2015). Qudsia *et al.* (2019) reported that soil *Rhizobium* can transform soil organic P into inorganic P under the action of ALP. The relative abundance of *Rhizobium* belonging to Proteobacteria in soil under the HPw treatment was significantly higher than in soil under the HPwm treatment (Fig. 2-D) and was positively correlated with the ALP content (Fig. 6-A). Studies have shown that inoculation with PSB such as *Bacillus, Rhizobium, Pseudomonas*, and *Micrococcus* increases P solubilization and crop yields (Liu *et al.* 2015; Cheng *et al.* 2019). Organic P is mineralized with phosphatase, which is released by PSB and catalyzes the hydrolysis of phosphate ester, thereby releasing phosphates (Novo *et al.* 2018). Increasing P fertilization is likely to suppress the activities of *Bacillus* and ACP in soils with high Olsen-P content, so inorganic P instead of organic P becomes the main source of P for microbes (Fig. 2-D; Table 4). Our results indicated that the soil water-soluble P concentration of HPw becomes similar to that of HPwm at the V12 stage, when maize requires the most P, due to the interaction between microbes and phosphatase (Table 3; Fig. 5). Future research should investigate the contributions of the microbial community composition and plant roots to the phosphatase activity. Simultaneously, to better understand the specific source of phosphatase in soil and its role in the soil phosphorus transformation mechanism, the association of phosphatase

activity with the expression of the *phoD* and *olpA* genes encoding these enzymes should be investigated using advanced techniques such as quantitative polymerase chain reaction.

5. Conclusion

The reduced P fertilization scheme that involves the application of P fertilizer only to wheat effectively reduced the P fertilizer input while maintaining a high maize yield in the wheat–maize rotation system at high residual P accumulation. P availability during the maize season was enhanced by the relatively high temperature, moisture, and frequent dry–wet alternating environment, which could be attributed mainly to effective microbial activities. Reduced P application resulted in enhanced ALP activity and increased abundances of *Bacillus* and *Rhizobium*, which could potentially ensure soil P availability. Future research should focus on further elucidating the biogeochemistry of P and the control of P availability. A reasonable reduction in P fertilization application in soils with high Olsen-P could balance the P level required for optimal crop growth, minimize the accumulation of soil P, and save P resources, which might be a sustainable agricultural practice strategy for the wheat–maize rotation system.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (41977019), the National Key Research and Development Program of China (2017YFD0200201 and 2017YFD0200706), the Shandong Key Research and Development Program, China (2019GNC106011), and the Provincial Agricultural Science and Technology Park Construction Project, Shandong (LKZ2018143). We thank Dr. Ashok Alva, Courtesy Professor, University of Florida, USA for reviewing the paper.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendices associated with this paper are available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

References

Ahmad M, Ahmad M, Hel-Naggar A, Rausman A, Abduljabbar A, Vithanage M, Elfaki J, Al-Faraj A, Al-Wabel M. 2018. Aging effects of organic and inorganic fertilizers on phosphorus fractionation in a calcareous sandy loam soil. *Pedosphere*, **28**, 873–883.

Andry A, Tiphaine C, Dominique M, Herintsitohaina R, Tantely R. 2019. Land management modifies the temperature sensitivity of soil organic carbon, nitrogen and phosphorus dynamics in a Ferralsol. *Applied Soil Ecology*, **138**, 112–122.

Arenberg M R, Arai Y. 2018. Uncertainties in soil physicochemical factors controlling phosphorus mineralization and immobilization processes. *Advances in Agronomy*, **154**, 153–200.

Chen W, Teng Y, Li Z G, Liu W X, Ren W J, Luo Y M, Christie P. 2018. Mechanisms by which organic fertilizer and effective microbes mitigate peanut continuous cropping yield constraints in a red soil of south China. *Applied Soil Ecology*, **128**, 23–34.

Chen X D, Jiang N, Condron L M, Dunfield K E, Chen Z H, Wang J K, Chen L J. 2019. Soil alkaline phosphatase activity and bacterial *phoD* gene abundance and diversity under long-term nitrogen and manure inputs. *Geoderma*, **349**, 36–44.

Cheng X K, Ji X X, Ge Y Z, Li J J, Qi W Z, Qiao K. 2019. Characterization of antagonistic *Bacillus methylotrophicus* isolated from rhizosphere and its biocontrol effects on maize stalk rot. *Phytopathology*, **109**, 571–581.

CRGCST (Cooperative Research Group on Chinese Soil Taxonomy). 2001. *Chinese Soil Taxonomy*. Science Press, Beijing and New York. pp. 166–167.

Da Cruz D F, Bortoletto-Santos R, Guimarães G G F, Polito W L, Ribeiro C. 2017. Role of polymeric coating on the phosphate availability as a fertilizer: insight from phosphate release by castor polyurethane coatings. *Journal of Agricultural and Food Chemistry*, **65**, 5890–5895.

Denef K, Six J, Bossuyt H, Frey S D, Paustian K. 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biology & Biochemistry*, **33**, 1599–1611.

Devkota M, Martius C, Lamers J P A, Sayre K D, Devkota K P, Vlek P L G. 2013. Tillage and nitrogen fertilization effects on yield and nitrogen use efficiency of irrigated cotton. *Soil & Tillage Research*, **134**, 72–82.

Feng K, Wang S, Wei Z Y, Wang Z J, Deng Y. 2020. Niche width of above- and below-ground organisms varied in predicting biodiversity profiling along a latitudinal gradient. *Molecular Ecology*, **29**, 1890–1902.

Han Y Y, Zhou S, Chen Y H, Kong X Z, Xu Y, Wang W. 2014. The involvement of expansins in responses to phosphorus availability in wheat, and its potentials in improving phosphorus efficiency of plants. *Plant Physiology and Biochemistry*, **78**, 53–62.

Islam M R, Chauhan P S, Kim Y, Kim M, Sa T. 2011. Community level functional diversity and enzyme activities in paddy soils under different long-term fertilizer management practices. *Biology and Fertility of Soils*, **47**, 599–604.

Jenkinson B J, Franzmeier D P. 2006. Development and evaluation of iron-coated tubes that indicate reduction in soils. *Soil Science Society of America Journal*, **70**, 183.

Jia X C, Liu P, Lynch J P. 2018. Greater lateral root branching density in maize improves phosphorus acquisition from

low phosphorus soil. *Journal of Experimental Botany*, **69**, 4961–4970.

Khan A, Lu G, Ayaz M, Zhang H, Wang R, Lv F, Yang X, Sun B, Zhang S. 2018. Phosphorus efficiency, soil phosphorus dynamics and critical phosphorus level under long-term fertilization for single and double cropping systems. *Agriculture Ecosystems & Environment*, **256**, 1–11.

Khan M S, Zaidi A, Ahmad E. 2014. Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. In: Khan M S, Zaidi A, Musarrat J, eds., *Phosphate Solubilizing Microorganisms*: *Principles and Application of Microphos Technology*. Springer International Publishing, Switzerland. pp. 31–62.

Li H, Huang G, Meng Q, Ma L, Yuan L, Wang F, Zhang W, Cui Z, Shen J, Chen X, Jiang R, Zhang F. 2011. Integrated soil and plant phosphorus management for crop and environment in China. A review. *Plant and Soil*, **349**, 157–167.

Li J, Li Z A, Wang F M, Zou B, Chen Y, Zhao J, Mo Q F, Li Y W, Li X B, Xia H P. 2015. Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. *Biology and Fertility of Soils*, **51**, 207–215.

Li M, Guo X H, Zhang M, Wang X P, Zhang G D, Tian Y C, Wang Z L. 2010. Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (*Zea mays* L.). *Plant Science*, **178**, 454–462.

Li S, Shakoor A, Wubet T, Zhang N L, Liang Y, Ma K P. 2018. Fine-scale variations of fungal community in a heterogeneous grassland in Inner Mongolia: Effects of the plant community and edaphic parameters. *Soil Biology & Biochemistry*, **122**, 104–110.

Li W, Feng J, Kwon K D, Kubicki J D, Phillips B L. 2010. Surface speciation of phosphate on boehmite (γ-AlOOH) determined from NMR spectroscopy. *Langmuir*, **26**, 4753–4761.

Liang J L, Liu J, Jia P, Yang T T, Zeng Q W, Zhang S C, Liao B, Shu W S, Li J T. 2020. Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *ISME Journal*, **14**, 1600–1613.

Liu Z G, Li Y C, Zhang S A, Fu Y Q, Fan X H, Patel J S, Zhang M. 2015. Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. *Applied Soil Ecology*, **96**, 217–224.

Lu D J, Song H, Jiang S T, Chen X Q, Wang H Y, Zhou J M. 2019. Managing fertiliser placement locations and source types to improve rice yield and the use efficiency of nitrogen and phosphorus. *Field Crops Research*, **231**, 10–17.

Lu R K. 1998. *Soil–Plant Nutrients Principles and Fertilization*. Chemical Industry Press, Beijing. pp. 153–208. (in Chinese)

Lu R K. 2000. *The Analysis Method of Soil Agro-Chemistry*. Chinese Agricultural Academic Press, Beijing. (in Chinese)

Luo G W, Ling N, Nannipieri P, Chen H, Raza W, Wang M, Guo S W, Shen Q R. 2017. Long-term fertilisation regimes affect the composition of the alkaline phosphomonoesterase encoding microbial community of a vertisol and its derivative soil fractions. *Biology and Fertility of Soils*, **53**, 375–388.

Macdonald G K, Bennett E M, Potter P A, Ramankutty N. 2011. Agronomic phosphorus imbalances across the world's croplands. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 3086–3091.

Macdonald N W, Zak D R, Pregitzer K S. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. *Soil Science Society of America Journal*, **59**, 233–240.

MOA (Ministry of Agriculture China). 2013. *China Agricultural Yearbook*. China Agricultural Publishing House, Beijing. pp. 206–222. (in Chinese)

Novo L A, Castro P M, Alvarenga P, da Silva E F. 2018. Plant growth-promoting rhizobacteria-assisted phytoremediation of mine soils. In: Prasad M N V, de Campos Favas P J, Maiti S K, eds., *Bio-Geotechnologies for Mine Site Rehabilitation*. Elsevier, Amsterdam. pp. 281–295.

Oliveira J R G, Silva E M, Teixeira-Rios T, Melo N F, Yano-Melo A M. 2015. Response of an endangered tree species from Caatinga to mycorrhization and phosphorus fertilization. *Acta Botanica Brasilica*, **29**, 94–102.

Pabis A, Caroline S K L. 2016. Promiscuity and electrostatic flexibility in the alkaline phosphatase superfamily. *Current Opinion in Structural Biology*, **37**, 14–21.

Qudsia Y, Saba K, Hamid R, Muhammad S K, Shahzad S, Muhammad A. 2019. *In silico* structural and functional characterization and phylogenetic study of alkaline phosphatase in bacterium, *Rhizobium leguminosarum* (Frank 1879), *Computational Biology and Chemistry*, **83**, 107142.

Ragot S A, Kertesz M A, Bünemann E K. 2015. Diversity of the *phoD* alkaline phosphatase gene in soil. *Applied Environmental Microbiology*, **81**, 7281–7289.

Sharpley A. 1985. Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Science Society of America Journal*, **49**, 905–911.

SSS (Soil Survey Staff). 2010. *Keys to Soil Taxonomy*. 11th ed. USDA-Natural Resources Conservation Service, Washington, D.C.

Song T, Xu F Y, Yuan W, Zhang Y J, Liu T Y, Chen M X, Hu Q J, Tian Y, Xu W F, Zhang J H. 2018. Comparison on physiological adaptation and phosphorus use efficiency of upland rice and lowland rice under alternate wetting and drying irrigation. *Plant Growth Regulation*, **86**, 195–210.

Sun D S, Bi Q F, Li K J, Dai P B, Yu Y, Zhou W W, Lv T, Liu X P, Zhu J, Zhang Q C, Jin C W, Lu L L, Lin X Y. 2017. Significance of temperature and water availability for soil phosphorus transformation and microbial community composition as affected by fertilizer sources. *Biology and Fertility of Soils*, **54**, 229–241.

Suseela V, Conant R T, Wallenstein M D, Dukes J S. 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Global Change Biology*, **18**, 336–348.

Tang X, Ma Y B, Hao X Y, Li X Y, Li J M, Huang S M, Yang X Y. 2009. Determining critical values of soil Olsen-P for maize and winter wheat from long-term experiments in China.

Plant and Soil, **323**, 143–151.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, dit Frey N F, Gianinazzi-Pearson V, Gilbert L B, Handa Y, Herr J R, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers P J, Masclaux F G, Murat C, *et al.* 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 20117–20122.

Turan M, Gulluce M, Von Wiren N, Sahin F. 2012. Yield promotion and phosphorus solubilization by plant growth-promoting rhizobacteria in extensive wheat production in Turkey. *Journal of Plant Nutrition and Soil Science*, **175**, 818–826.

Turner B L, Haygarth P M. 2001. Phosphorus solubilization in rewetted soils. *Nature*, **411**, 258–258.

Wang Y, Zhao X, Guo Z Y, Jia Z J, Wang S Q, Ding K. 2018. Response of soil microbes to a reduction in phosphorus fertilizer in rice-wheat rotation paddy soils with varying soil P levels. *Soil & Tillage Research*, **181**, 127–135.

Wang Y, Zhao X, Wang L, Jin S Z, Zhu W B, Lu Y Y, Wang S Q. 2016a. A five-year P fertilization pot trial for wheat only in a rice-wheat rotation of Chinese paddy soil: Interaction of P availability and microorganism. *Plant and Soil*, **399**, 305–318.

Wang Y, Zhao X, Wang L, Wang Y J, Li W, Wang S Q, Xing G X. 2015. The regime and P availability of omitting P fertilizer application for rice in rice/wheat rotation in the Taihu Lake Region of southern China. *Journal of Soils and Sediments*, **15**, 844–853.

Wang Y, Zhao X, Wang L, Zhao P H, Zhu W B, Wang S Q. 2016b. Phosphorus fertilization to the wheat-growing season only in a rice–wheat rotation in the Taihu Lake region of China. *Field Crops Research*, **198**, 32–39.

Wei X M, Hu Y J, Razavi B, Zhou J, Shen J L, Nannipieri P, Wu J S, Ge T. 2019. Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. *Soil Biology & Biochemistry*, **131**, 62–70.

Xia X Y, Zhang P P, He L L, Gao X X, Li W J, Zhou Y Y, Li Z X, Li H, Yang L. 2019. Effects of tillage managements and maize straw returning on soil microbiome using 16S rDNA sequencing. *Journal of Integrative Plant Biology*, **61**, 765–777.

Xu J, Liu S J, Song S R, Guo H L, Tang J J, Yong J W H, Ma Y D, Chen X. 2018. Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. *Soil Biology & Biochemistry*, **120**, 181–190.

Xue Y D, Xin J S, Ren Y, Wan G H, Zhang G L, Yang R R. 2015. Characteristics and gradations of cultivated land fertility for winter wheat-summer maize rotation system in North China. *Journal of Agricultural Resources and Environment*, **32**, 530–536. (in Chinese)

Yuan Y Y, Gao M G, Zhang M X, Zheng H H, Zhou X W, Guo Y, Zhao Y, Kong F M, Li S S. 2017. QTL mapping for phosphorus efficiency and morphological traits at seedling and maturity stages in wheat. *Frontiers in Plant Science*, **8**, 614.

Zhang L, Peng Y, Zhou J C, George T S, Feng G. 2020. Addition of fructose to the maize hyphosphere increases phosphatase activity by changing bacterial community structure. *Soil Biology & Biochemistry*, **142**, 107724.

Zhang M, Alva A K, Li Y C, Calvert D V. 2001. Aluminum and iron fractions affecting phosphate solubility and reactions in selected sandy soils. *Soil Science*, **166**, 940–948.

Zhang M, Li C L, Li Y C, Harris W G. 2014. Phosphate minerals and solubility in native and agricultural calcareous soils. *Geoderma*, **232–234**, 164–171.

Zhao Z B, He J Z, Geisen S, Han L L, Wang J T, Shen J T, Wei W X, Fang Y T, Li P P, Zhang L M. 2019. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome*, **7**, 1–16.

Zheng W K, Liu Z G, Zhang M, Shi Y F, Zhu Q, Sun Y, Zhou H Y, Li C L, Yang Y C, Geng J B. 2017. Improving crop yields, nitrogen use efficiencies, and profits by using mixtures of coated controlled-released and uncoated urea in a wheat-maize system. *Field Crops Research*, **205**, 106–115.

Executive Editor-in-Chief ZHANG Wei-li
Managing Editor SUN Lu-juan