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Effect of straw retention and mineral fertilization on P speciation and P-transformation microorganisms in water extractable colloids of a Vertisol

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Abstract

Water extractable colloids (WECs) serve as crucial micro particulate components in soils, playing a vital role in the cycling and potential bioavailability of soil phosphorus (P). Yet, the underlying information regarding soil P species and P-transformation microorganisms at the microparticle scale under long-term straw retention and mineral fertilization is barely known. Here, a fixed field experiment (~13 years) in a

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Vertisol was performed to explore the impacts of straw retention and mineral fertilization on inorganic P, organic P and P-transformation microorganisms in bulk soils and WECs by sequential extraction procedure, P K-edge X-ray absorptions near-edge structure (XANES), ^{31}P nuclear magnetic resonance (NMR), and metagenomics analysis. In bulk soil, mineral fertilization led to increases in the levels of total P, available P, acid phosphatase (ACP), high-activity inorganic P fractions (Ca_2-P, Ca_8-P, Al-P, and Fe-P) and organic P (orthophosphate monoesters and orthophosphate diesters), but significantly decreased the abundances of P cycling genes including P mineralization, P-starvation response regulation, P-uptake and transport by decreasing soil pH and increasing P in bulk soil. Straw retention had no significant effects on P species and P-transformation microorganisms in bulk soils but brought increases for organic carbon, total P, available P concentrations in WECs. Furthermore, straw retention caused greater change in P cycling genes between WECs and bulk soils compared with the effect of mineral fertilization. The abundances of *phoD* gene and *phoD*-harbouring *Proteobacteria* in WECs increased significantly under straw retention, suggesting that the P mineralizing capacity increased. Thus, straw retention could potentially accelerate the turnover, mobility and availability of P by increasing the nutrient contents and P mineralizing capacity in microscopic colloidal scale.

Keywords: water extractable colloids, inorganic P, organic P, P-cycling genes, straw retention, mineral fertilization

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1. Introduction

Phosphorus (P) has a vital function in the productivity of agroecological system (Jiang et al., 2015). Vertisol (Staff, 2010), also known as a Shajiang black soil in Chinese Soil Taxonomy, covers approximately 4×10^6 hectares in the Huang-Huai-Hai Plain of China (Guo et al., 2022). The characteristics of the Vertisol contain abundant calcium, scant organic matter, and poor fertility (Chen et al., 2020). The strong P fixation capacity by abundant calcium and poor supply capacity of P restrict agricultural production severely (Ma et al., 2019). Straw retention and mineral fertilization are commonly employed to enhance soil nutrient contents in this area (Zhao et al., 2018). Under mineral fertilization and straw retention, Ca_2-P, Fe-P and Al-P contents increased, but Ca_{10}-P concentration reduced, thereby promoting the transformation of P fractions (Xu et al., 2022). Cao et al. (2022) suggested that the combination of straw retention and mineral fertilization significantly increased both inorganic and organic P species concentrations. Crop straw, which is rich in organic matter and contains a certain amount of nitrogen (N), P, and other nutrients, has demonstrated potential effects on the cycling and processing of P (Damon et al., 2014).

The assessment of potential bioavailability and mobility of soil P heavily relies on the speciation and distribution of P in soil aggregates (Ranatunga et al., 2013). Agricultural management practices like the application of fertilizer and straw could modify the microhabitat's physicochemical environment through their influence on soil aggregation (Ju et al., 2023). Maize straw promoted the accumulation and

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stabilization of inorganic and organic P in soil aggregates, particularly in the 250–2000 μm fraction. Additionally, it decreased the relative contribution rates of the <53 μm fraction to inorganic and organic P fractions compared with mineral fertilizer (Cao et al., 2021). Generally, soil aggregate fractionation contains the particle size of > 0.25 mm, 0.053-0.25 mm, and <0.053 mm, and the distribution and dynamics of P in these aggregates have been widely researched (Cheng et al., 2019; Deng et al., 2021). However, there are few studies on the forms and distribution of P in soil water-extractable colloids (WECs; <2 μm in size), which significantly contribute to P cycling due to the large binding ability, high mobility and bioavailability of P (Fresne et al., 2022; Jiang et al., 2023). WECs, readily extracted upon water contact, are regarded as indexes of mobile soil colloids (Missong et al., 2018) and main factors that impact the mobility and availability of soil P (Zhang et al., 2021). Colloidal P could contribute to plant-available P as reported by Montavo et al. (2015). Additionally, the microaggregates (including colloidal size fractions) provided a favorable habitat for microorganisms and the biochemical processes functioning at the microparticle scale would be also important for soil P cycling and availability (Totsche et al., 2018). However, the information related to how straw retention and mineral fertilization managements affect soil P dynamics at scales of WECs remains scarce.

Microorganisms are instrumental in facilitating the transformation of soil P species, P cycling and P availability regulation (Bergkemper et al., 2016). The processes of microbial P transformation primarily consists of: (1) inorganic P solubilization (e.g., *gcd*); (2) organic P mineralization (e.g., *phoD, phoA, phy*);

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(3) P starvation response regulation (e.g., *phoR*, *phoB*); and (4) P uptake and transport system (e.g., *pst*) (Richardson and Simpson, 2011). Fertilization could further change the abundance and taxonomic assignments of P cycling gene clusters (Dai et al., 2020; Zhang et al., 2023). For example, continuous N fertilization over an extended period may lead to a decline in soil pH, inhibition of microbial growth, alterations in the composition of the microbial community, and ultimately the reduction in the capacity for P solubilization (Rousk et al., 2010). Additionally, genes expression related to organic P mineralization, P-starvation regulation, P-uptake and transport are primarily affected by the environmental P supply (Hsieh and Wanner, 2010). Several researches have shown that the adequate P supply inhibited the genes expression associated with P-starvation response (e.g. *phoR*), as well as genes encoding alkaline phosphatase (e.g. *phoD*) and phytase (e.g. *phy*) (Yao et al., 2018; Xie et al., 2020). Straw retention could bring the increase in soil organic C, potentially enhancing the diversity and richness of *phoD*-harboring microbes and the *phoD* abundance (Cao et al., 2022). Moreover, alterations in the P transformation genes are driven by the structural effects of soil aggregates in addition to P availability (Neal et al., 2017). However, little is known about the richness and distribution of genes related to P transformation in WECs fraction with the treatments of straw retention and mineral fertilization, which will offer a new perspective on P cycling and availability from a microbial perspective.

The long-term field experiments (~13 years) under straw retention and mineral fertilization were conducted. This study aims to: (1) investigate the responses of P speciation, P-cycling-related genes and

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taxonomic assignments in bulk soils and WECs under straw retention and fertilization management strategies; (2) explore the relationship between P species, P-transformation genes and soil properties. Finally, these results could elucidate the underlying mechanisms of soil P cycling and availability under mineral fertilization and straw retention from the microparticle and microbial perspective, providing an important insight into regulating P cycling in agriculture soils.

2. Materials and methods

2.1 Experimental design

In 2008, a field trial was conducted in Mengcheng County (33°9′N, 116°32′E), Anhui Province, China, to investigate the rotation of winter wheat and summer maize. The soil is classified as a Vertisol (Staff, 2010), which is derived from fluvio-lacustrine sediments. The region experiences an average annual temperature and precipitation of 14.8℃ and 732.6 mm respectively.

Six treatments with three replicates (each plot area was 43.2 m^2) were carried out: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilization (W1M0F1), (5) both wheat and maize straw retention without fertilization (W1M1F0), and (6) a combination of both wheat and maize straw retention with mineral fertilization (W1M1F1). In the W0M1F1 treatment, maize straw was chopped into fragments approximately 10 cm in length and uniformly distributed in each plot after harvest, while

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wheat straw was removed. In the W1M0F1 treatment, wheat straw was similarly returned to plots and maize straw was removed. For W1M1F0 and W1M1F1 treatments, maize and wheat straw are both returned to plots when they are harvested. The amounts of residue incorporation for wheat and maize were 7500 and 12000 kg/ha respectively. For the fertilization treatments (i.e., W0M0F1, W0M1F1, W1M0F1, W1M1F1), 240.0 kg/ha N (55% as basal fertilizer and 45% as topdressing during the reviving-jointing period), 90.0 kg/ha P, and 90.0 kg/ha K (100% as basal fertilizer) were applied in each growing season of winter wheat. The 300.0 kg/ha N (50% as basal fertilizer and 50% as topdressing at the flare opening period), 90.0 kg/ha P and 90.0 kg/ha K (100% as basal fertilizer) were applied in each growing season of summer maize. The fertilizers comprised of compound and urea fertilizer (N-P_2O_5-K_2O: 15-15-15). The contents of P in maize straw and wheat straw was about 1.5 and 0.8 g/kg respectively (Chai et al., 2021). In addition, weeds, disease, and pest control for both wheat and maize were consistent.

2.2 Soil sampling and water extractable colloids (WECs)

The soil samples with six treatments were conducted after wheat harvest in June 2021. Five soil cores (0–20 cm) were gathered from each replicate plot using the quincunx sampling method, and then blended evenly to create a composite sample. The divisions of three subsamples were made for each sample. The first subsample was preserved at 4 ◦C to examine P (MBP) and microbial biomass C (MBC), along with the acid and alkaline phosphatase activities (ACP and ALP). Another sample was at stored −80 °C for metagenomics analysis. For other soil chemical properties test, the last sample was subjected to air-

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drying, grinding, and subsequently sieving through a 2 mm mesh. In this study, the soil fraction consisting of particles smaller than 2 mm was designated as bulk soil.

To further investigate the impact the sole straw retention and sole mineral fertilization on P cycling in soil colloids, the particle-size fractionation method following Stokes' Law (Sequaris and Lewandowski, 2003) was utilized to obtain WECs for the W0M0F0, W0M0F1 and W1M1F0 treatments in this study. About 113-116 g of moist soil samples (equivalent to 100 g of dry soil) was blended with 200 mL ultrapure water, and then shaken at a speed of 150 rpm for a duration of 6 h. Afterward, we added an extra 600 mL of ultrapure water and blended thoroughly. The particles >20 μm were allowed to settle for a period of 6 min. The 2-20 μm was then obtained by eliminating the supernatant following an addition sedimentation of 12 h. The final supernatant containing colloidal particle fraction (<2 μm) was obtained and defined as WECs. The proportions of particles with >20 μm, 2-20 μm and <2 μm to bulk soil were shown in Fig. S1.

2.3 Soil chemical properties

A pH meter was utilized to measure soil pH. An elementary analyzer was utilized for soil organic carbon (SOC), and total nitrogen (TN). After microwave digestion, total P concentrations (TP) were gained by inductively coupled plasma optical emission spectroscopy (ICP-OES). Available P (AP, Olsen-P) concentration was quantified by Olsen and Sommers (1982).

The chloroform fumigation method outlined by Vance et al. (1987) and Brookes et al. (1982) was utilized

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to quantify the soil MBC and MBP. The extracted C with 0.5 M K_2SO_4 in non-fumigated and fumigated samples was determined with the Multi N/C 2100S TOC-TN analyzer. The dissolved organic carbon (DOC) was quantified as the extracted organic C by K_2SO_4 extract from the non-fumigated samples (Wu et al., 2019). MBC was quantified by measuring the variation in extractable C content between the non-fumigated and fumigated soil samples, using the universal conversion factor of 0.45. MBP was calculated as the variation in extractable P with 0.5 M $NaHCO_3$ between the non-fumigated and fumigated soil samples, with a conversion factor of 0.40. The measurement of ACP and ALP followed the procedures outlined by Tabatabai and Bremner (1969).

2.4 Phosphorus sequential extraction procedure and P K-edge XANES spectroscopy

The modified sequential extraction procedure, as described by Jiang and Gu (1989) and Audette et al. (2016), was utilized to extract various P fractions including Ca_2-P, Ca_8-P, Al-P, Fe-P, occluded-P (O-P) and Ca_{10}-P in bulk soils. Then the method outlined by Murphy and Riley (1962) was utilized to ascertain the concentration of each P fraction.

P K-edge X-ray absorptions near-edge structure (XANES) spectra were utilized to clarify the P bonding fractions in WECs, and acquired at Beamline 4B7A of the Beijing Synchrotron Radiation Facility, Beijing, China. Dibasic calcium phosphate dihydrate (DCP, $CaHPO_4{\cdot}2H_2O$), hydroxyapatite (HAP, $Ca_5(PO_4)_3OH$), aluminum phosphate (Al-P, $AlPO_4$), iron phosphate dihydrate (Fe-P, $FePO_4{\cdot}2H_2O$) and inositol hexakisphosphate (IHP) were chosen as references. For XANES data collection, P references

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and soil samples were thinly spread on the carbon tape with a P-free, double-sided in PFY mode with a SiLi detector. Multiple spectra were obtained with three duplicates for each sample and then averaged. The spectra were studied using Athena (0.9.26) with the energy calibration at 2149 eV (E0), aligning with the peak position of $AlPO_4$, as described by Beauchemin et al. (2003). Then, we performed the Linear combination fitting (LCF) within the energy range spanning from −10 eV to 30 eV relative to E0, and the goodness of fit was determined based on the chi-squared and R values. The most likely P species considered was considered based on these results. The P K-edge XANES spectra of P reference compounds were as shown in Fig. S2.

2.5 Solution ^{31}P NMR spectroscopy

Solution ^{31}P-NMR spectroscopy were performed to clarify P species (Turner, 2008). The 1 g bulk soil and WECs sample was mixed with 10 mL of 0.25 M NaOH and 0.05 M Na_2EDTA and shaken for 4 h to extract P (Cade-Menun and Liu, 2014; Jiang et al., 2017). The procedure was outlined in our prior study (Bai et al., 2023). The ^{31}P-NMR spectra were acquired using a Bruker 500-MHz spectrometer with 4.32 s relaxation delay, 0.68 s acquisition time, 5000 scans, and 90° pulse width(Cade-Menun et al., 2010). Compound identification relied on their chemical shifts following the calibration of the orthophosphate peak to 6.0 ppm (Table S1). To validate peak identification, samples were spiked with *myo*-inositol hexakisphosphate, α- and β- glycerophosphate, as well as adenosine monophosphate (Fig. S3). Instead of being classified as monoesters, the α- and β-glycerophosphate as well as mononucleotides (Glyc+nucl)

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were categorized as orthophosphate diesters (Doolette et al., 2009). Integration was conducted on spectra with broadening at 7 and 2 Hz to calculate the area under each peak. To quantify the concentrations of P species, the peak areas were multiplied by the concentration of NaOH-Na_2EDTA extractable P. The spectra of bulk soil and WECs were processed using MestReNova 10.0.2 software, as shown in Fig. S4.

2.6 DNA extraction and metagenomics analysis

The process of soil DNA extraction was carried out with a FastDNA Spin kit (MP Biomedicals, USA). The Agilent 5400 was utilized to determine the purity, integrity and concentration of the extracted DNA. The generation of sequencing libraries was carried out using the NEBNext® Ultra™ DNA Library Prep Kit (PerkinElmer, USA). For each sample, barcodes were incorporated to enable sequence attribution. After end-polished, A- tailing, and adapter ligation, the DNA fragments were subsequently subjected to PCR amplification. Finally, a NovaSeq 6000 instrument was utilized for sequencing, generating paired-end reads. Reads containing low-quality bases and N base were removed (Hua et al., 2015).

MEGAHIT was used to assemble the filtered reads (fastq formats) by *de Bruijn* graph with the minimum k-mer size of 21 (Li et al., 2015). The default settings of Prodigal were used to identify the protein-coding genes, as described by Hyatt et al. (2010). For functional annotation, we employed the Diamond software to align the identified genes against the nonredundant protein sequences database of NCBI and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases following the methodologies as outlined by Kanehisa and Goto (2000), Buchfink et al. (2015) and Huson et al. (2016).

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According to the prior studies of Bergkemper et al. (2016), a cumulative of 29 genes associated with P-transformation were identified, along with their corresponding KO numbers. These genes were categorized into four distinct groups: (1) genes associated with inorganic P-solubilization; (2) genes associated with organic P-mineralization; (3) genes associated with P-starvation regulation, and (4) genes associated with microbial P-uptake and transport. Table S2 provides a comprehensive list of the categorized genes along with their names, function descriptions, and KEGG Orthology (KO) numbers. The sequence data have been submitted in the NCBI Sequence Read Archive (PRJNA909638).

2.7 Statistical analysis

The IBM SPSS and R software were utilized for statistical analyses and data visualization. The normality distribution (Shapiro–Wilks tests) were performed before ANOVA. To identify significant differences among mean values at a significance level of 0.05, the Tukey's honestly significant differences (HSD) test was employed. The differences of soil properties, total P, inorganic P, organic P, ACP, and ALP between bulk soils and WECs were tested by independent-samples T test. The differences of P cycling genes composition in bulk soils and WECs were displayed by principal component analysis (PCA). Principal coordinate analysis (PCoA) was utilized to present the microbial bacterial β-diversity for typical P-solubilization (*gcd*) and mineralization (*phoD*) genes. The associations between the abundances of P-transformation genes and soil characteristics were assessed using Spearman's correlations with the correlation coefficients (R) > 0.6 and P-value <0.05. Structural equation modeling (SEM) was used to

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explore the relationships among agricultural managements, soil properties, and P-cycling related genes by Amos (24.0). The model fit was assessed with goodness of fit (GFI) and root square mean error of approximation (RMSEA).

3. Results

3.1 Soil properties in bulk soils and WECs

Straw retention incorporated with mineral fertilization (i.e., W0M1F1, W1M0F1, W1M1F1) decreased soil pH by 1.76-1.89 units and alkaline phosphatase activity (ALP) by 160.25-183.37 μg/(g·h) significantly, but increased significantly organic C by 2.66-4.73 g/kg, total N by 0.36-0.60 g/kg, total P by 0.17-0.19 g/kg, available P by 28.11-31.97 mg/kg, and acid phosphatase activity (ACP) by 174.12-449.25 μg/(g·h), respectively compared with the control treatment (i.e., W0M0F0) (Table 1). The variations primarily resulted from the utilization of mineral fertilizers, as there were no noteworthy distinctions observed in these parameters between straw retention combined with mineral fertilization treatments and sole mineral fertilizer (i.e., W0M0F1). The application of straw retention (i.e., W1M1F0) had little effect on these soil properties except for slight increases in soil MBC and MBP contents compared with the control treatment. The outcomes suggested mineral fertilization showed more prominent impact on soil characteristics compared to that of straw retention. Mineral fertilization indeed enhanced soil nutrient contents, but caused soil acidification. The soil acidification was not effectively alleviated under straw returning combined with mineral fertilization.

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The WECs accounted for 9.73-11.05% of bulk soils, and the proportions of WECs were not affected by mineral fertilization and straw retention (Fig. S1). The significantly higher concentrations of SOC, TN, TP and available P were monitored in WECs than those in bulk soils for all the tested samples including the control treatment (i.e., W0M0F0), sole mineral fertilization (i.e., W0M0F1) and sole straw retention (i.e., W1M1F0) (Fig. 1 A-D). The influence of either mineral fertilization or straw retention on physicochemical properties of WECs was more obvious than that on bulk soils. For example, organic C and total N contents in WECs experienced a substantial rise following the implementation of straw retention compared with the control treatment from Fig. 1 A and B.

3.2 P bonding fractions in bulk soils and WECs

The concentrations of total inorganic P and Ca_2-P, Ca_8-P, Al-P, and Fe-P under straw retention incorporated with mineral fertilization increased remarkably by 128.93-146.99 mg/kg, 15.41-17.30 mg/kg, 3.19-4.38 mg/kg, 59.74-68.97 mg/kg, and 44.08-54.46 mg/kg, respectively compared with the control as shown in Table 2. Accordingly, the marked increases in the proportion of Ca_2-P, Ca_8-P, Al-P, and Fe-P were observed, while the proportion of Ca_{10}-P decreased remarkably (Fig. S4). These differences were mainly caused by mineral fertilization. There was also no significant difference between straw retention incorporated with mineral fertilization and sole mineral fertilization. The straw retention had little impact on the concentrations of each inorganic P fraction compared with the control.

According to the XANES analysis of WECs, there were notable increases in the proportions of Al-P and

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Fe-P, but remarkable decreases in the proportions of DCP and IHP was observed after mineral fertilization compared with the control treatment (Table 3 and Fig. S5). However, the straw retention brought slight increases in the proportions of Fe-P and IHP.

3.3 Solution ^{31}P NMR analysis of bulk soils and WECs

The concentrations and proportion of orthophosphate in bulk soils increased by 146.4-182.6 mg/kg and 18.6-21.3% significantly under straw retention incorporated with mineral fertilization compared with the control and sole straw retention treatments (Table 4 and Fig. S6A). Organic P concentrations also increased under mineral fertilization, among which orthophosphate monoesters and orthophosphate diesters increased by 12.78-27.00 mg/kg and 7.55-10.05 mg/kg, respectively. Furthermore, the concentration of each P specie in bulk soil showed no notable difference between straw retention incorporated with mineral fertilization treatments and sole mineral fertilization treatment (Table 4). In comparison with the control, the concentration of orthophosphate monoesters and orthophosphate diesters in bulk soil increased slightly under sole straw retention, but this difference was not statistically significant. These results manifested that the effect of mineral fertilization on P species concentration was more apparent than that of straw retention.

Notably, the concentrations of orthophosphate, orthophosphate monoesters, orthophosphate diesters, and Glyc+nucl (i.e., α/β-glycerophosphate and mononucleotides) in WECs were significantly greater (~2.5 times) than those in bulk soil for all the tested samples (Table 4 and 5). Mineral fertilization had more

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significant effects on the concentrations of P species in WECs compared with those in bulk soils. Relative to the control, the concentrations of orthophosphate, orthophosphate monoesters and orthophosphate diesters rise sharply after mineral fertilization for WECs, while the significant increase of only orthophosphate concentrations was detected for bulk soils. Furthermore, the concentration of these P species in WECs under sole straw retention increased slightly in comparison with the control (Table 5).

3.4 Genes associated with P transformation in bulk soils and WECs

In bulk soils, there were remarkable decreases in total relative abundances of genes associated with P-transformation under the combined application of straw retention and mineral fertilization compared with the control. These genes included those related to organic P-mineralization (e.g., *phoA*, *phoD*, *phy*, *ugpQ*), P-starvation regulation (e.g., *phoR*), P-uptake and transport (e.g., *phnCDE*) as described in Figs. 2A and B. No notable difference was observed in the abundances of these P transformation genes in bulk soils between straw retention combined with mineral fertilization and sole mineral fertilization, but they were significantly different from those for sole straw retention. This indicated that the decrease in abundances of P transformation genes was mainly caused by mineral fertilization but not by straw retention. Correspondingly, the PCA results also revealed clear separations for the genes related to P-cycling between with (i.e., W0M0F1, W1M0F1, W0M1F1, and W1M1F1) and without (i.e., W0M0F0 and WM1F0) mineral fertilization treatments (Fig. 3 A).

The PCA analysis (Fig. 3 B) exhibited a clear segregation between the P-cycling genes in WECs and

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those in bulk soils for all the tested samples, including sole mineral fertilization, sole straw retention and the control treatments. Straw retention caused significant differences of relative abundance for many gene species including *ppa*, *ppk*, *phoD*, *phoN*, *phy*, *phoR*, *phnCDE* and *ugpBAEC* between WECs and bulk soils. In contrast, sole mineral fertilization caused significant differences of less gene species including *gcd*, *ppx*, *glpABCK* and *phoR*, and the control treatment caused significant differences of *glpABCK* and *phoR* genes (Fig. 4 B). These results suggested that straw retention caused greater change of P cycling gene between WECs and bulk soils compared with mineral fertilization.

3.5 Taxonomic assignments of *phoD* and *gcd* genes

The *phoD* gene (encoding alkaline phosphatases) and *gcd* gene (encoding glucose dehydrogenase for synthesizing) serve as critical indicators of P mineralization and solubilization, respectively. As shown in Fig. 4, straw retention caused significant increase of the abundance for *phoD* gene and mineral fertilization caused significant decrease of the abundance for *gcd* genes in WECs compared with bulk soils. Thus, we further performed the taxonomic assignments of *phoD* and *gcd* genes.

For bacterial taxa containing the *phoD* gene in WECs (Fig. 5 A), the abundance of *Proteobacteria* increased significantly under sole straw retention when compared to those in bulk soils. For bacterial taxa containing the *gcd* gene in WECs (Fig. 5 B), the abundance of *Acidobacteria* decreased significantly compared with those in bulk soils under mineral fertilization. Additionally, the bacterial β-diversity in WECs showed a clear divergence from those in bulk soils for all the treatments (Fig. S7).

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3.6 Correlations between P-cycling genes and soil properties, P species in bulk soils and WECs

According to Spearman's Rank correlations (Fig. S8), more P gene species were correlated with soil properties and nutrients in bulk soils than WECs ($R > 0.6$, $P < 0.05$), suggesting that the response of P cycling genes to soil properties in bulk soil were more sensitive than those in WECs. Specially, a correlation was detected between the majority of P cycling genes and soil nutrients including C, N, P in bulk soils. Whereas, there was no consistent trends in WECs.

According to Fig. 6, mineral fertilization influenced the P-cycling genes by decreasing soil pH and increasing total P in bulk soil. The model fit in bulk soil was : GFI=0.939, RMSEA=0.036. Furthermore, the decrease in soil pH affected positively the genes involved in organic P mineralization (0.82, $P < 0.01$) and the increase in total P had negative effect on the genes involved in P-starvation regulation (-0.77, $P < 0.01$). In WECs, agricultural managements affected the P-cycling genes by increasing total P (0.98, $P < 0.01$) and organic C (0.92, $P < 0.01$).The model fit in WECs was : GFI=0.964, RMSEA=0.000. Moreover, total P had negatively affected the genes related to and organic P mineralization (-0.67, $P < 0.01$) and inorganic P solubilization (-0.69, $P < 0.05$).

4. Discussions

4.1 Response of soil properties, P species and transformation genes in bulk soils

In bulk soil, mineral fertilization decreased soil pH, increased soil TP, thus decreasing the abundances of P transformation genes. Soil acidification might be due to the increased protons release from nitrification

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processes occurring under mineral N fertilization (Guo et al., 2010). The significant increases in TP concentrations under mineral fertilization might be closely associated to the enhanced organic matter from crop residues and the input of P fertilizers (Zhang et al., 2018). Moreover, Tong et al. (2019) reported that mineral fertilization also increased root exudates, which brought the increases in soil organic matter and nutrients.

Generally, the P mineralization, P-starvation regulation, P-uptake and transport genes were primarily influenced by the environmental availability of P (Hsieh and Wanner, 2010; Richardson and Simpson, 2011). Under conditions of low soil P, microorganisms exhibited an upregulation of genes within the *Pho* regulon, specifically those encoding phosphatases and phosphate transporters (Vershinina and Znamenskaya, 2002). The expression of *phoR* and *phoD* was governed by the presence of P starvation conditions (Xie et al., 2020). The phytase was inhibited by high level of phosphate (Yao et al., 2018) and higher abundance of *phy (3-phytase)* was observed in P-deficient soils compared to P-rich soils (Siles et al., 2022). The *ugpQ* gene also usually accumulated in P starvation conditions as the operon of glycerophosphodiester-utilizing system (Luo et al., 2009). Therefore, in the control and straw retention treatments with lower P concentrations, higher abundances of *phoD*, *phy*, *phoR*, and *ugpQ* genes were observed in comparison with the mineral fertilization treatments (Fig. 2). Mineral fertilization reduced the abundance of genes about P mineralization (e.g., *phoA*, *phoD*, *phy*, *ugpQ*), P-starvation regulation (e.g., *phoR*), P-uptake and transport (e.g., *phnCDE*) significantly (Fig. 2). Consistent with our findings,

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prior research has indicated that a notable decline in the *phoD* gene abundance with mineral fertilization alone or combined with maize straw compared with the control (Ikoyi et al., 2018). Long-term P application resulted in a reduction in the abundances of *phoR* gene according to Dai et al. (2020). Additionally, observed changes in soil pH significantly impacted microbial abundances and communities (Neal et al., 2017; Wan et al., 2021). According to Chen et al. (2017), soil pH was identified as the primary factor exerting an influence on the microbial community compositions harboring the *phoD* gene, with a positive correlation observed between the soil pH and the abundance of the *phoD* gene. Studies have provided evidence that a decrease in soil pH could inhibit bacterial/fungal growth (Li et al., 2020), modify the microbial community compositions (Rousk et al., 2010), and decrease the relative abundances of *Actinobacteria* and *Proteobacteria* for *phoD* gene (Luo et al., 2017), which in turn decreases P mineralization capacity.

According to the Spearman's Rank correlations in this study, the *phoD*, *phoA*, *phy*, *ugpQ*, and *phoR* genes abundances were correlated negatively with the contents of orthophosphate, orthophosphate monoesters, orthophosphate diesters, and positively with soil pH ($p < 0.05$) (Fig. S8 A). Thus, the decline in the abundance of the P-cycling related genes can be attributed to increasing soil P contents and low soil pH under mineral fertilization.

In bulk soil, straw retention showed no significant impact on soil properties, P species and transformation genes. Straw decomposition was affected by the composition of straw (e.g., the C/N, C/P, lignin, cellulose

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of straw) and soil characteristics (e.g., soil aeration, pH and nutrient contents). The high C/N, lignin, and cellulose in wheat and maize straw might slow down straw decomposition (Talbot and Treseder, 2012). The C/N in wheat and maize straw (52-73:1) were significantly higher than suitable microorganisms C:N (25-30:1) for straw decomposition (Cai et al., 2018), and microorganisms needed to consume soil original N when decomposing straw. Therefore, the straw retention without N addition could limit the decomposition rate of straw. Thus, the straw retention for 13 years did not show any significant impact on soil C, N, P nutrients. Yet it is noteworthy that although the decomposition rate of straw was slow, it started to have slight effects on the accumulation of soil microorganisms C and P in bulk soils and was expected to have a more obvious effect in the longer term. The slow decomposition of straw provided the nutrients and promoted crop root exudation, consequently fostering the growth of soil microbial and augmenting soil MBC (Wang et al., 2021). The slight increase in MBC derived the increase of MBP (Spohn and Kuzyakov, 2013). When N and P fertilizers were added, straw retention incorporated with mineral fertilization could enhance microbial activity, improve soil microbial C/N and C/P, promote straw decomposition and increase organic C contents (Li et al., 2018). The input of N and P fertilizers brought the significant increase in soil N and P contents (Zhang et al., 2018). In this study, straw retention incorporated with mineral fertilization had remarkable influences on soil characteristics and nutrients, which was significantly different from sole straw retention. There was no discernible disparity in soil pH between straw retention incorporated with mineral fertilization and single mineral fertilization, indicating

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that straw retention did not alleviate soil acidification caused by mineral fertilization.

4.2 Response of soil properties, P species and transformation genes in WECs

The higher concentrations of SOC, TN, TP, AP and P species in WECs compared with bulk soil (Fig. 1) indicated that nutrients within WECs are enriched, which was because of their high specific surface area (Jiang et al., 2014). The influences of mineral fertilization and straw retention on soil properties and P species in WECs were stronger compared with those in bulk soils, suggesting that the physicochemical properties of soil microparticles were more sensitive than bulk soil in response to soil environmental disturbance. Soil colloids are the most active constituent, representing the micro particulate phase of soils, and play a fundamental role in the cycling of P (Fresne et al., 2022). Previous studies demonstrated that colloids were the important vectors governing P mobility and bioavailability (Rick and Arai, 2011). According to de Jonge et al. (2004), colloidal P can make a substantial contribution to the transportable P, amounting to as much as 75% in arable soils. More inorganic and organic P accumulated in the WECs compared with bulk soils (Tables 4 and 5), which could improve the potential bioavailability and mobility of P (Krause et al., 2020). Notably, although the practice of straw retention did not result in any significant changes on nutrient contents in bulk soils, it brought significant increases in TN and SOC contents (Fig. 1 A and B) and slight increases in the concentrations of TP and each P species for WECs. This indicated that straw retention promoted the accumulation of nutrients on WECs, which exerted a considerable influence on the supply and cycling of P.

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Straw retention caused the greater change of P cycling genes between WECs and bulk soils compared with mineral fertilization (Fig. 4 B) and led to a significant increase of *phoD* gene in WECs compared with bulk soils. Research conducted by Fierer et al. (2012) and Ling et al. (2014) suggested that higher concentrations of total N, P and organic C could favor the growth of microorganisms. For bacterial taxa containing *phoD* gene, the abundance of *Proteobacteria* (Fig. 5 A) increased significantly in WECs compared with those in bulk soils under sole straw retention. This indicated that straw retention might increase the *phoD* gene abundance by influencing *phoD*-harbouring *Proteobacteria*, and then increase P mineralizing capacity in WECs. Several studies have highlighted that *Proteobacteria* has been recognized as a crucial group of microorganisms involved in the mineralization of P (Zhang et al., 2023) and the increase in *phoD*-harbouring *Proteobacteria* could improve potential P mineralization (Xie et al., 2020). The *Proteobacteria* belongs to copiotrophic microorganisms groups, and accumulates in rich nutrient soils (Wang et al., 2022). In our research, the notable increases in SOC, TN and each P specie in WECs were likely to provide favorable conditions of copiotrophic bacteria (e.g., *Proteobacteria*) under straw retention. Generally, the WECs (clay particles, <2 μm) including natural organic matter (e.g., humus) and inorganic colloids (silicate, and Al/Fe oxides) (Zhang et al., 2021) were considered to be the best natural microorganism adsorbents (Zhao et al., 2014; Madumathi, 2017). Previously conducted research has indicated that most bacteria (65%) associated with <2 μm soil particulates (Oliver et al., 2007). The population of the bacteria (*Pseudomonas putida*) attached to the clay particle in Red soil

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(*Ultisol*) was significantly higher compared to the populations found on silt and sand particles (Wu et al., 2012). Furthermore, the increased SOC could improve the surface area and activity of WECs (Zhao et al., 2014), thus increasing microorganism adhesion (Van Gestel et al., 1996). SOC was a key component of P binding in colloids (Sun et al., 2023). Thus, we considered that the P cycling microorganisms in soil colloids might be influenced mainly by the increased nutrients contents.

In this study, although mineral fertilization also caused the enhancements of SOC contents in WECs, it brought dramatical increase of P contents and decrease of pH by 1.76-1.89 units, which restricted the abundance of P cycling genes in both WECs and bulk soils as discussed before. Therefore, the difference of P-cycling genes between WECs and bulk soil under mineral fertilization was less significant than those under straw retention. Additionally, the consistent change trends of the *gcd* gene and *gcd*-harbouring *Acidobacteria* indicated that the decrease in *gcd* gene abundance in WECs might be driven by the *gcd*-harboring *Acidobacteria* under mineral fertilization. (Khan et al., 2007), the *gcd* gene coding the membrane-bound quinoprotein glucose dehydrogenase (PQQGDH) was involved in the regulation of the process of making inaccessible mineral P soluble, such as some rock phosphate, hydroxyapatite, and Ca phosphates. Wu et al. (2021) have shown that the increase in *gcd*-harbouring *Acidobacteria* improved P solubilization. The *Acidobacteria* was acidophilic and oligotrophic bacteria. Most of their members lived in low nutrient or high acidity environments. The abundance of *Acidobacteria* was often negatively correlated with soil nutrient contents and pH (Jones et al., 2009; Rousk et al., 2010). As

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mentioned above, soil pH decreased significantly (Table 1) and this might lead to the increase of *Acidobacteria* in bulk soils after mineral fertilization. The WECs had strong soil buffering capacity by the exchangeable ion, organic C and clay particles (Curtin and Trolove, 2013; Dvorackova et al., 2022), and could alleviate the pH change, which did not support the growth of *Acidobacteria*. The pH buffering capacity and greater nutrient contents in WECs might limit the expression of *Acidobacteria* compared with bulk soils under mineral fertilization, thus causing the significant decrease in *gcd* gene abundance in WECs compared with the bulk soil.

5. Conclusions

This study provides systematic insights into P speciation and P transformation microorganisms at the soil microparticle scale (WECs) compared with bulk soil under straw retention and mineral fertilization. Straw retention caused more obvious impact on the accumulation of organic C and total N of WECs and the greater change of P cycling genes between WECs and bulk soils even than mineral fertilization. The significant increase in the abundance of gene encoding for alkaline phosphatase (*phoD*) and *phoD*-harbouring *Proteobacteria* for WECs compared with bulk soils indicated the improved P mineralization capacity of WECs under straw retention. This information provided strong evidences that straw retention could potentially affect the turnover, mobility and availability of P mainly by changing the physicochemical and biochemical processes involved in the P transformation of soil colloids.

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Declaration of competing interest

The authors declare no competing interests.

Supplementary material

Supplementary material associated with this paper are available on the online version.

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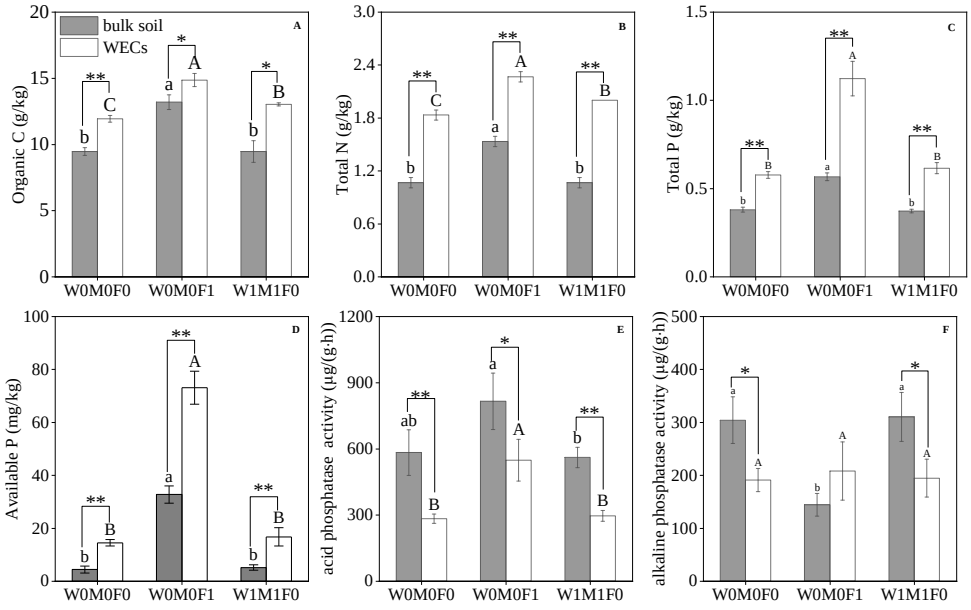

Fig.1 Soil properties in bulk soil and water-extractable colloids (WECs) for the W0M0F0, W0M0F1, W1M1F0 treatments

A: Soil organic carbon (SOC), B: Total nitrogen (N), C: Total phosphorus (P), D: Available phosphorus (P), E: acid phosphatase activity (ACP), F: alkaline phosphatase activity (ALP). Significant differences between treatments in bulk soil are indicated by lowercase letters ($p<0.05$). Significant differences between treatments in WECs (< 2μm) are indicated by capital letters ($p<0.05$). Significant differences between bulk soil and WECs are as follows, * $p < 0.05$ and ** $p < 0.01$ (Independent-samples T test).

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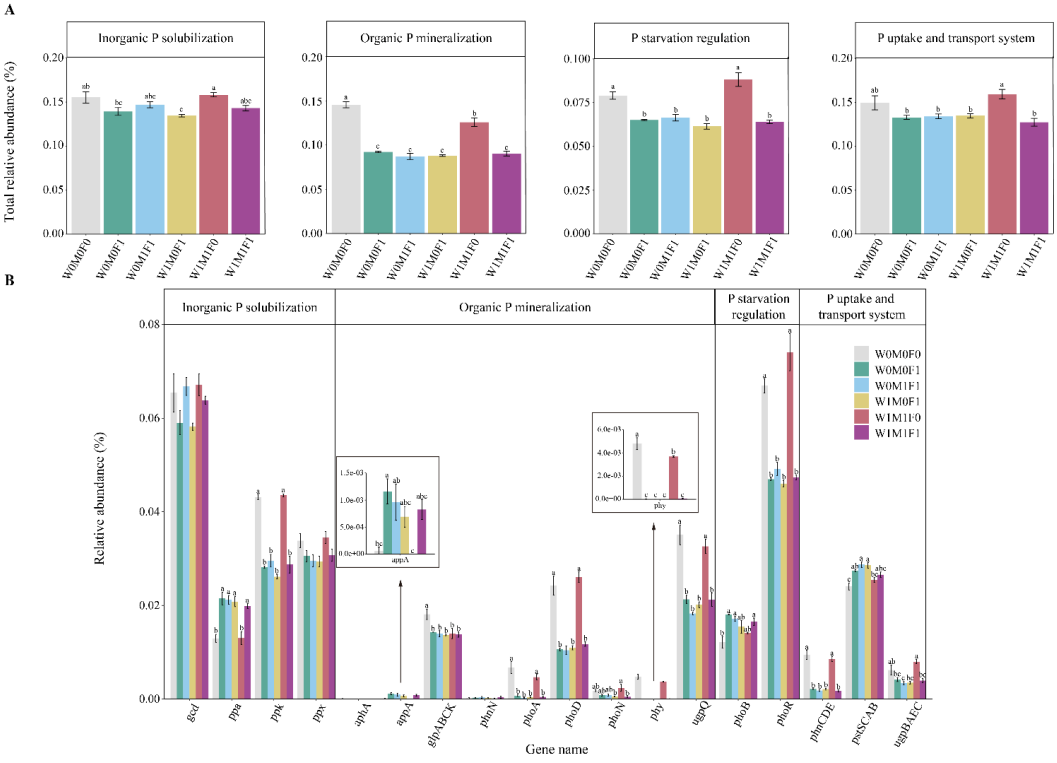

Fig. 2 Relative abundance of representative genes responsible for microbial (1) inorganic P solubilization, (2) organic P-mineralization, (3) P-starvation regulation, and (4) P-uptake and transport in bulk soil

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. The relative abundances of genes were calculated related to the annotated reads. Significant differences between treatments in bulk soil are indicated by lowercase letters ($p<0.05$). The relative abundance of glp transporter systems was calculated as the average abundances of gene glpA, glpB, glpC, and glpK; the phn transporter systems was calculated as the average abundances of gene phnC, phnD, and phnE; the pst transporter systems was calculated as the average abundances of gene pstS, pstC, pstA, and pstB; The ugp transporter systems was calculated as the average abundances of gene ugpB, ugpA, ugpE, and ugpC.

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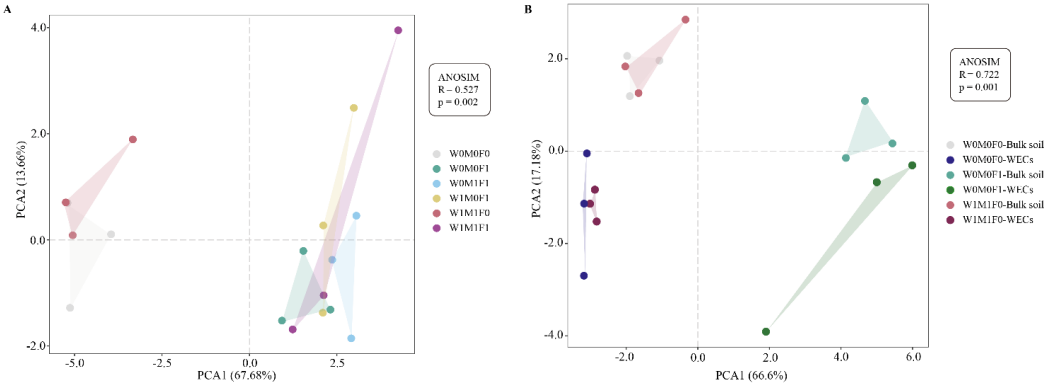

Fig. 3 Principal component analysis (PCA) of P-transformation gene composition in bulk soil (A) and water-extractable colloids (WECs, B)

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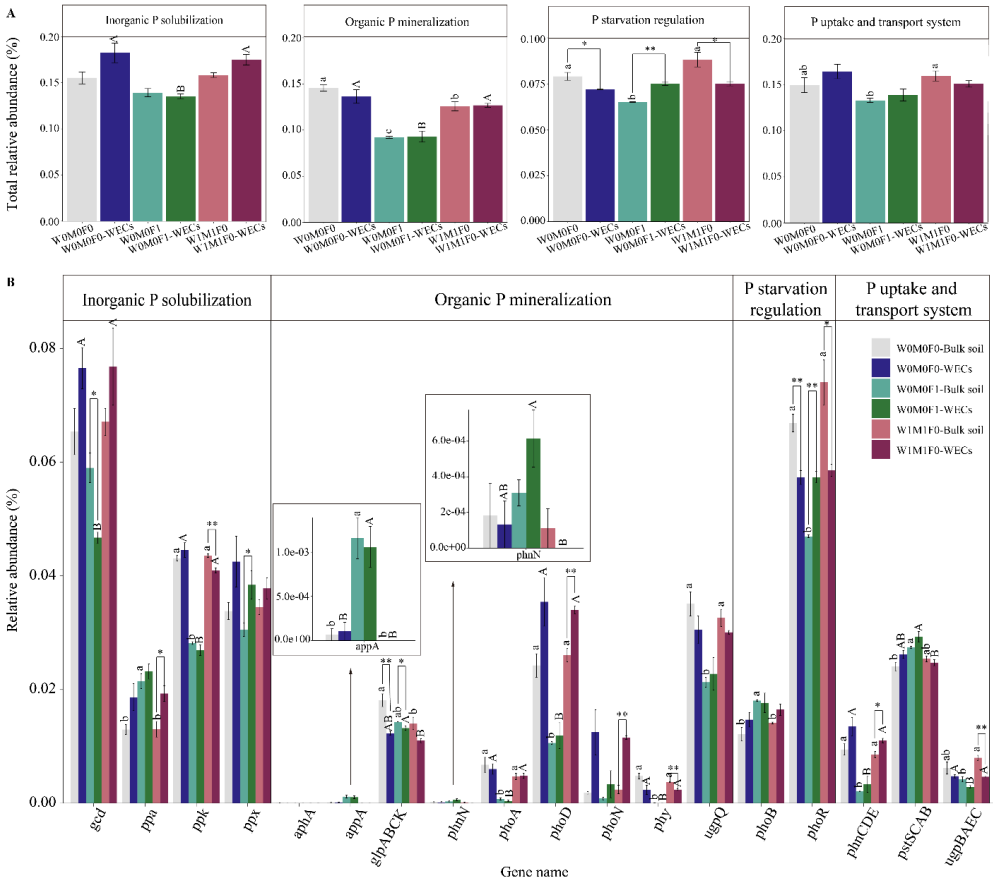

Fig. 4 Relative abundance of representative genes responsible for microbial (1) inorganic P solubilization, (2) organic P-mineralization, (3) P-starvation regulation, and (4) P-uptake and transport in bulk soils and water-extractable colloids (WECs) among the W0M0F0, W0M0F1, and W1M1F0 treatments

The relative abundances of genes were calculated related to the annotated reads. Significant differences between treatments in bulk soil are indicated by lowercase letters ($p<0.05$). Significant differences between treatments in WECs (< 2μm) are indicated by capital letters ($p<0.05$). Significant differences between bulk soil and WECs are as follows, * $p < 0.05$ and ** $p < 0.01$ (Independent-samples T test). The relative abundance of glp transporter systems was calculated as the average abundances of gene glpA, glpB, glpC, and glpK; the phn transporter systems was calculated as the average abundances of gene phnC, phnD, and phnE; the pst transporter systems was calculated as the average abundances of gene pstS, pstC, pstA, and pstB; The ugp transporter systems was calculated as the average abundances of gene ugpB, ugpA, ugpE, and ugpC.

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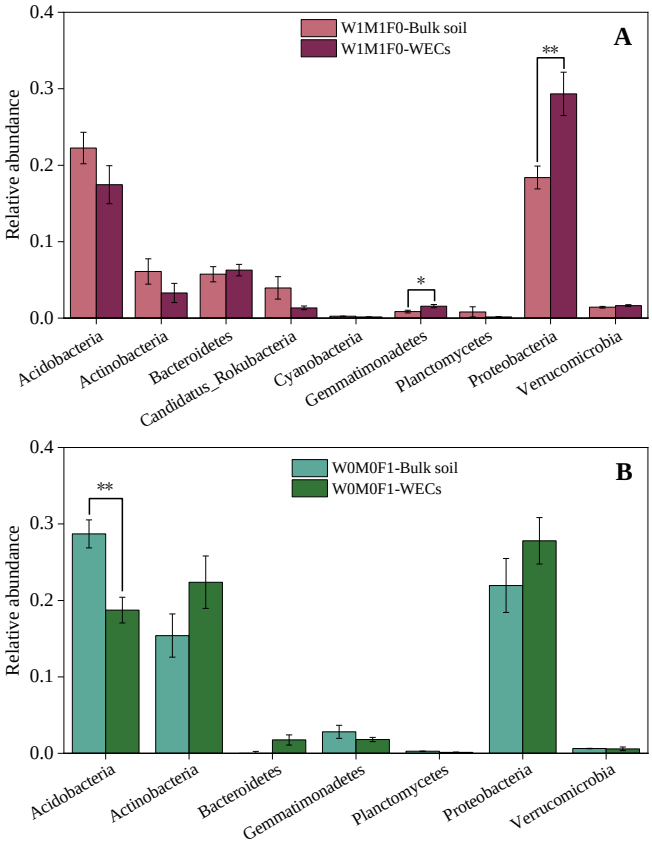

Fig. 5 Taxonomic assignments of phoD gene for the W1M1F0 treatment (A) and gcd gene for the W0M0F1 treatment (B) at the phylum level in bulk soil and water-extractable colloids (WECs)

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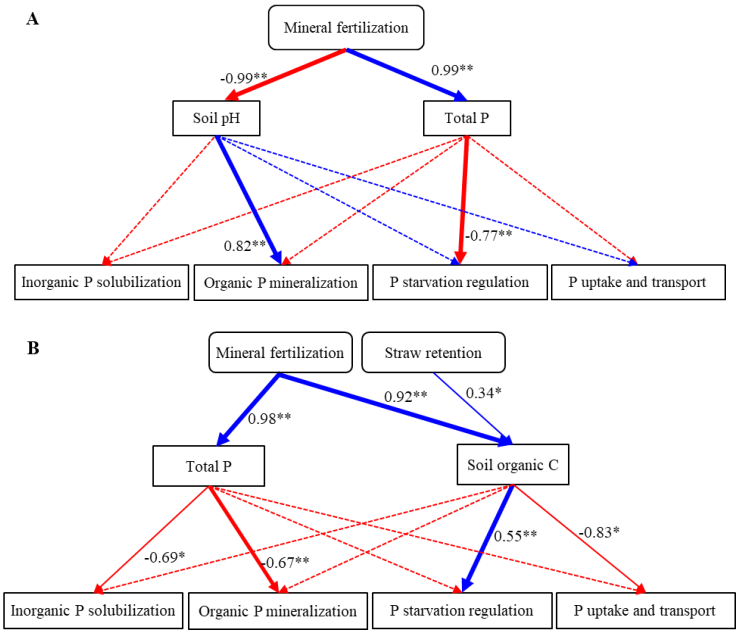

Fig. 6. Structural equation model (SEM) showing the relationship among mineral fertilization and straw retention, soil properties, and P cycling-related gene in bulk soil (A) and water-extractable colloids (WECs, B).

The blue and red solid arrows represent the significant positive and negative relationships between different variables. The dashed arrows represent nonsignificant relationships. The numbers near the blue and red arrows are the path coefficients. * , $P < 0.05$; * *, $P < 0.01$.

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Table 1 Soil properties of bulk soil among six treatments

Soil properties	W0M0F0	W0M0F1	W0M1F1	W1M0F1	W1M1F0	W1M1F1
pH	6.90±0.07a	5.10±0.14b	5.06±0.09b	5.14±0.08b	6.79±0.08a	5.01±0.31b
Gravimetric moisture (%)	0.14±0.01a	0.15±0.01a	0.14±0.01a	0.15±0.01a	0.15±0.02a	0.15±0.01a
Soil organic C (g/kg)	9.47±0.29c	13.20±0.56ab	12.13±0.74b	13.70±0.56ab	9.47±0.81c	14.20±0.96a
Total N (g/kg)	1.07±0.06c	1.53±0.06ab	1.43±0.06b	1.67±0.15a	1.07±0.06c	1.57±0.06ab
Total P (g/kg)	0.38±0.01b	0.57±0.02a	0.56±0.04a	0.55±0.03a	0.37±0.01b	0.56±0.01a
Available P (mg/kg)	4.43±1.34b	32.77±3.26a	32.54±3.18a	36.40±1.35a	5.18±1.04b	32.49±4.12a
Microbial biomass P (mg/kg)	6.80±0.44a	nd	nd	nd	9.01±4.35a	nd
Dissolved organic C (mg/kg)	54.21±2.56b	133.43±2.80a	142.03±8.13a	134.11±3.97a	57.01±9.61b	140.01±9.51a
Microbial biomass C (mg/kg)	316.39±59.52a	357.95±24.32a	343.28±90.16a	307.96±27.45a	336.23±52.37a	387.89±21.52a
Acid phosphatase activity (μg/(g·h))	582.80±103.58c	815.06±128.42abc	756.92±142.48bc	1032.05±149.59ab	506.63±46.11c	1102.26±133.11a
Alkaline phosphatase activity (μg/(g·h))	304.01±43.97a	144.08±21.39b	120.64±88.90b	138.34±12.14b	310.30±46.22a	143.76±44.88b

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. Values are means ± standard error. The "nd" indicates that the microbial biomass P were not detected. Significant differences between treatments are indicated by the different lowercase letters ($p<0.05$).

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Table 2 Concentrations (mg/kg) of inorganic P fractions in bulk soil

Samples	Ca_2-P	Ca_8-P	Al-P	Fe-P	O-P	Ca_{10}-P	Total inorganic P
W0M0F0	3.39±0.17b	1.27±0.22b	25.14±1.29b	27.46±3.86b	37.31±3.02c	119.95±4.70a	214.53±2.93c
W0M0F1	20.39±2.83a	5.58±0.64a	90.23±8.03a	71.54±5.20a	44.91±2.18abc	119.04±3.11a	351.69±14.93a
W0M1F1	18.80±0.45a	4.46±1.04a	84.88±13.86a	72.13±4.98a	46.34±4.35abc	116.85±6.13a	343.46±22.74a
W1M0F1	19.87±5.24a	5.19±0.65a	94.11±15.81a	81.92±8.76a	48.11±3.08ab	112.32±12.05a	361.52±23.06a
W1M1F0	3.19±0.56b	1.20±0.31b	22.76±0.90b	25.99±2.70b	41.13±2.52bc	111.17±8.09a	205.44±2.78c
W1M1F1	20.69±3.57a	5.65±0.81a	83.91±3.61a	79.95±5.52a	54.36±5.84a	110.18±14.65a	354.74±21.09a

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. Inorganic P fractions includes calcium-bound P (Ca-P), aluminum-bound P (Al-P), iron-bound P (Fe-P), and occluded phosphate (O-P), Ca-P can be divided into dicalcium phosphate (Ca_2-P), octacalcium phosphate (Ca_8-P) and apatite (Ca_{10}-P). Values in each column followed by the different lowercase letters indicate significant differences ($P < 0.05$).

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Table 3 Phosphorus K-edge XANES fitting results (%) showing the relative percent of each P species in water-extractable colloids (WECs)

Samples	DCP	Al-P	Fe-P	IHP
W0M0F0	29.25±2.36a	20.46±0.93b	23.69±2.51b	26.60±1.09a
W0M0F1	7.31±0.93b	31.35±0.53a	44.55±1.42a	16.79±0.49b
W1M1F0	23.91±4.14a	20.14±1.98b	28.58±2.28b	27.37±0.70a

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. DCP, dibasic calcium phosphate dihydrate (DCP, $CaHPO_4 \cdot 2H_2O$); Al-P, aluminum phosphate ($AlPO_4$); Fe-P, iron phosphate dihydrate ($FePO_4 \cdot 2H_2O$); and IHP, inositol hexakisphosphate, Values in each column followed by the different lowercase letters indicate significant differences ($P < 0.05$).

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Table 4 Concentrations (mg/kg) of P species in bulk soil evaluated in the solution ^{31}P NMR analysis

Samples	NaOH-Na_2EDTA extracted P	Inorganic P		Organic P					
		Orth	Pyro	Orthophosphate monoesters				Orthophosphate diesters	
				Monoesters	Myo-IHP	Scyllo-IHP	Other mono	Diesters	Glyc+nucl
W0M0F0	120.47±11.00b	62.26±0.23c	5.60±0.02a	41.40±1.17b	7.16±0.47a	1.56±0.45a	32.68±2.08a	11.21±0.92b	10.59±0.92a
W0M0F1	309.62±30.41a	221.21±4.47ab	7.73±1.41a	61.94±1.25ab	13.27±0.27a	4.42±0.09a	44.24±0.89a	18.76±4.31ab	16.57±1.23a
W0M1F1	320.30±32.89a	225.11±12.29ab	5.67±1.90a	68.27±10.58a	11.26±0.61a	4.50±0.25a	52.51±11.44a	21.26±3.61a	19.09±0.55a
W1M0F1	340.18±40.35a	244.85±7.47a	7.35±0.22a	68.40±8.30a	12.14±6.55a	3.70±1.84a	52.56±3.59a	19.59±0.60ab	18.39±2.29a
W1M1F0	126.11±14.31b	60.78±0.62c	6.39±1.35a	44.67±0.83b	7.90±0.08a	2.43±0.02a	34.33±0.94a	14.28±1.14ab	11.54±0.74a
W1M1F1	286.84±29.14a	208.68±5.37b	5.20±1.34a	54.18±4.51ab	9.41±1.72a	4.17±0.11a	40.6±6.33a	18.78±0.48ab	17.72±1.02a

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. Calculation by including diester degradation products (i.e. Glyc+nucl: α/β- glycerophosphate, and mononucleotides) with orthophosphate diesters (Diesters) rather than orthophosphate monoesters (Monoesters). Phosphorus compounds include orthophosphate (Orth), pyrophosphate (Pyro), myo inositol hexakisphosphate (Myo-IHP), scylloinositol hexakisphosphate (Scyllo-IHP), other monoesters not specifically identified (Other mono), α/β- glycer-ophosphate (Glyc), and mononucleotides (nucl). Values in each column followed by the different lowercase letters indicate significant differences ($P < 0.05$).

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Table 5 Concentrations (mg/kg) of P species in water-extractable colloids (WECs) evaluated in the solution ^{31}P NMR analysis

Samples	NaOH-Na_2EDTA extracted P	Inorganic P		Organic P						
		Orth	Pyro	Orthophosphate monoesters				Orthophosphate diesters		
				Monoesters	Myo-IHP	Scyllo-IHP	Other mono	Diesters	Glyc+nucl	DNA
W0M0F0	258.36±19.99b	96.97±12.00b	14.02±1.05a	110.24±6.77b	17.28±0.58a	4.32±0.15a	88.63±6.04b	37.14±6.29a	28.58±4.63a	0.97±0.12b
W0M0F1	777.38±76.78a	545.53±2.71a	21.82±0.11a	158.19±6.93a	13.63±3.79a	5.46±0.03a	139.10±3.17a	51.84±4.11a	30.01±4.01a	5.46±0.03a
W1M1F0	280.02±28.65b	111.96±9.46b	16.40±5.33a	110.56±10.38b	17.78±1.65a	4.48±0.38a	88.31±9.10b	41.09±4.42a	29.96±3.78a	1.12±0.09b

The six treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), (3) maize straw retention combined with mineral fertilization (W0M1F1), (4) wheat straw retention combined with mineral fertilizer (W1M0F1), (5) both wheat and maize straw retention with no fertilizer (W1M1F0), and (6) both wheat and maize straw retention combined with mineral fertilizer (W1M1F1) respectively. Calculation by including diester degradation products (i.e. Glyc+nucl: α/β- glycerophosphate, and mononucleotides) with orthophosphate diesters (Diesters) rather than orthophosphate monoesters (Monoesters). Phosphorus compounds include orthophosphate (Orth), pyrophosphate (Pyro), myo inositol hexakisphosphate (Myo-IHP), scylloinositol hexakisphosphate (Scyllo-IHP), other monoesters not specifically identified (Other mono), α/β- glycer-ophosphate (Glyc), and mononucleotides (nucl). Values in each column followed by the different lowercase letters indicate significant differences ($P < 0.05$).