

Response to Reviewer Comments

Responses to Reviewer 2:

Comments to the Author

This paper described the influences of straw retention and mineral fertilizer on the different phosphorous (P) forms in bulk soil and water-extractable colloid fractions, including the two P forms and their specific P species. The addition of P-related metagenomics analysis provides a comprehensive map of P cycling concerning different land management in the field. The results are very interesting to the current studies of P cycling in soils, with advanced technologies applied, which can help better understand P transformation in different fractions, but the paper could be more precise and would benefit from restructuring. The statistical methods you chose need to be further considered with respect to your observation size. The Result section is well written in general, but it would be better to modify some of the Figures and clarify some of your results. The major weakness of this paper, from my perspective, is the unclarification of your highlights, which means the Discussion section should be heavily revised. The bullet points in the Discussion section should be clearly delivered accordingly, a summarized paragraph is recommended for each subsection. Therefore, the recommendation for this manuscript is a major revision.

Response: *Thanks for your constructive suggestions on our paper. We have revised the paper according to your suggestions. The following is the answers and revisions we have made in response to the questions and suggestions on an item-by-item basis. A detailed explanation of the revision follows below.*

Comment # 1:

Material and methods

Line 102- Once you decided to use the abbreviation of all your six treatments, use them consistently for the rest of the manuscript

Response: *Thanks for your suggestion. We used the abbreviation of six treatments consistently for the rest of the manuscript.*

Comment # 2:

Line 131: Are there reasons why you only fractionate the 3 treatments?

Response: *To further investigate the impact the sole straw retention (W1M1F0) and sole mineral fertilization (W0M0F1) on P cycling in soil colloids (WECs), the two treatments and the control treatment (W0M0F0) were fractionated.*

Comment # 3:

Line 132: Why do you use 'moist soil samples' for sedimentation? Do you also measure the soil texture?

Response: *Thanks for your suggestion.*

We used the field-fresh soil samples for sedimentation. We have added the description in Line 132-135: The field-fresh soil samples were used for sedimentation to replicate natural conditions where soil exists in its natural state, neither completely dry or saturated, enabling a more accurate study of these natural processes.

We also measured the soil texture, and added the description in Line 140-142: The soil was classified as sandy loam according to the international soil texture classification standard. The mass proportions of particles with >20 µm, 2-20 µm and <2 µm to bulk soil were shown in Fig. S1.

Comment # 4:

Line 137: I think you mean 'The mass proportion of particles...'

Response: Yes. We have revised it.

Line 141-142: The mass proportions of particles with $>20\text{ }\mu\text{m}$, $2\text{-}20\text{ }\mu\text{m}$ and $<2\text{ }\mu\text{m}$ to bulk soil were shown in Fig. S1.

Comment # 5:

Line 140: Which method and in which soil/solution ratio (w/v) do you use for pH measurement? As far as I know, there are big differences between the CaCl_2 , H_2O_2 , and KCl methods. And which instrument do you use (specify all the instrument information you use for your analysis)?

Response: We measured soil pH in a 1:2.5 soil/ ultrapure water suspension with Rex Electric Chemical PHSJ-3F. We have added it in the manuscript.

Line 144-145: A pH meter (Rex Electric Chemical PHSJ-3F) was utilized to measure soil pH in a 1:2.5 soil/ ultrapure water suspension.

Comment # 6:

Line 140: Any pretreatment for SOC and TN measurements? The chemical measurements need to be briefly described (apply to the rest of the method section e.g. Line 145, Line 155-Line 156...)

Response: We have revised it as follows :

Line 146-148: Prior to measuring SOC and TN, the samples were passed through a 0.149mm sieve. For SOC measurement, 1M HCl was added to the samples in small increments until effervescence stops (Schumacher, 2002).

Line 153-159: 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 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.

Line 163-167: These fractions included $\text{Ca}_2\text{-P}$, extracted with 0.25 M NaHCO_3 (pH 8.0); $\text{Ca}_8\text{-P}$, extracted with 0.5 M NH_4Ac (pH 4.2); Al-P , extracted with 0.5 M NH_4F (pH 8.2); Fe-P , extracted with 0.1 M $\text{NaOH-Na}_2\text{CO}_3$ (pH 12.0); occluded-P (O-P), extracted with 0.3 M CD (sodium citrate-dithionite-sodium hydroxide, pH 13); and $\text{Ca}_{10}\text{-P}$, extracted with 0.25 M H_2SO_4 (pH 1.0).

Comment # 7:

Line 169: Reconsider two 'considered'...

Response: We deleted one 'considered' in Line 179-180.

Line 179-180: The most likely P species was considered based on these results.

Comment # 8:

Line 207: Specify the version of SPSS and R you use and cite the relevant references. For R, list the packages you use and find the relevant citations.

Response: It is a constructive suggestion. We have provided the version of SPSS and R as well as the package.

Line 218-232: The IBM SPSS (version 25.0) and R (version 4.2.0) software were utilized for statistical analyses

and data visualization. The normality distribution (Shapiro–Wilks test) 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) with the R package “FactoMineR” (Lê Sébastien et al., 2008). Principal coordinate analysis (PCoA) was utilized to present the microbial bacterial β -diversity for typical P-solubilization (gcd) and mineralization (phoD) genes with the R package “vegan” and “ape” (Paradis and Schliep, 2019; Oksanen J, 2024). The associations between the abundances of P-transformation genes and soil characteristics were assessed using Spearman's correlations by R package “psych” with the correlation coefficients (R) > 0.6 and P -value <0.05 (Revelle, 2024). Structural equation modeling (SEM) was used to 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).

Comment # 9:

Line 216: SEM and PCA may not be applicable considering the size of your observation. Please at least check the degree of freedom to see if your interpretation, especially SEM results, can be regarded as reliable and stable. Otherwise, there are also similar models for a smaller observation size.

Response: Thanks for your suggestion.

The degree of freedom (df) was 5.

The Chi-square/df was typically used to characterize good model fit. We have added it in Line 323-325: The Chi-square/df was 1.8 , which was less than 2 and indicated that the SEM model was a superior fit (Alavi et al., 2020).

Comment # 10:

Results

Line 231: (Table 1) I guess

Response: Yes. We have added Table 1.

Line 242-244: The application of sole 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 (Table 1).

Comment # 11:

Line 238: See above, either use the abbreviations or use the full name across the manuscript. And could you explain why you only measured the 3 treatments, not all of them? I also see differences from Table 1 between the 6 treatments and a significant increase in IP fractions, at least in W1M1F1, for example. Otherwise, I would think only include the 3 treatments in your results and discussion.

Response: Thanks for your suggestion. The abbreviations were used consistently throughout the manuscript.

To further explore the impact the sole straw retention (W1M1F0) and sole mineral fertilization (W0M0F1) on P cycling in soil colloids (WECs), these two treatments and the control treatment (W0M0F0) of water extractable colloids were fractionated and investigated.

We analyzed the soil properties, P fractions and genes associated with P transformation in bulk soils across six treatments. For water-extractable colloids (WECs), these indicators were analyzed among three treatments (W0M0F1, W1M1F0 and W0M0F0). Focusing on these three representative treatments allow us to study the

specific mechanisms and effects of straw retention/mineral fertilization alone on phosphorus cycling at a microscopic colloidal scale.

Significant increases in soil properties and IP fractions were observed under mineral fertilization treatments (W0M0F1, W0M1F1, W1M0F1, W1M1F1), with no significant differences among these treatments. We then further investigated the impact of mineral fertilization alone (W0M0F1) on phosphorus cycling in soil colloids (WECs).

Comment # 12:

Line 240: I personally would recommend avoiding overusing unclear words such as 'obvious', 'change', 'effect', 'little' etc., And better avoid 'for example' in the result section. You either present them or not.

Response: *We have revised it as suggested.*

Line 251-254: The influence of either mineral fertilization or straw retention on physicochemical properties of WECs was more remarkable than their effects on bulk soils. Organic C and total N contents in WECs experienced a substantial rise following the implementation of straw retention compared to the control treatment, as depicted in Fig. 1 A and B.

Comment # 13:

Line 283: 'This indicated that...' This, for me, looks like a Discussion, not a Result.

Response: *Thanks for your suggestion. The sentence has been deleted.*

Comment # 14:

Line 289: 'all the tested samples' make it clear, as you only fractionated 3 treatments, then better not use 'all'

Response: *OK, we have revised it as suggested.*

Line 298-299: The PCA analysis (Fig. 3 B) exhibited a clear segregation between the P-cycling genes in WECs and those in bulk soils for the W0M0F1, W1M1F0 and W0M0F0 treatments.

Comment # 15:

Line 290: 'Staw retention'. which treatment do you mean? sole straw retention?

Response: *OK, we have revised it as suggested.*

Line 299-301: Sole 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.

Comment # 16:

Line 293: 'The control treatment caused significant...' This also happened in the previous description. The control should perform as a reference, which means it is not a treatment but a benchmark you need to compare firstly with other treatments (e.g. mineral fertilizer, sole straw retention). The comparisons between other treatments (other than control) can be performed afterwards.

Response: *Thanks for your suggestion. We have deleted them.*

Comment # 17:

Line 299- Check grammar

Response: *We have checked grammar and revised it.*

Line 306-310: 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, sole straw retention significantly increased the abundance of the *phoD* gene, whereas mineral fertilization significantly decreased the abundance of the *gcd* gene in WECs compared with bulk soils.*

Comment # 18:

Line 310- Does this mean that the other fractions are more sensitive to P cycling genes ? Is it just because of the low proportion of WECs?

Response: *The more P gene species were correlated with soil properties and nutrients in bulk soils than WECs, then the P genes species were more likely to be affected by soil properties and nutrient content in bulk soils.*

This suggested that the response of P cycling genes to soil properties in bulk soil were more sensitive than those in WECs.

Comment # 19:

Fig. 1: This is quite interesting that straw retention does not increase the OM in total , but only changes the partitioning of OM in different fractions. While, the sole mineral fertilizer significantly increases the OM in total. How do you harvest the plants with sole mineral fertilizer treatment? Do you leave the roots or bottom stems in the field? Missing in the Method

Response: *For the sole mineral fertilizer treatment, straws were removed and the roots were left in the field. This information has been added in Line 112-113 of Materials and methods section.*

Line 112-113: For the W0M0F1 treatments, straws were removed and the roots were left in the field.

Comment # 20:

Fig. 2: Increase the text size of the plot and modify the note.

Response: *OK. We have increased the text size and modified the note in Fig.2.*

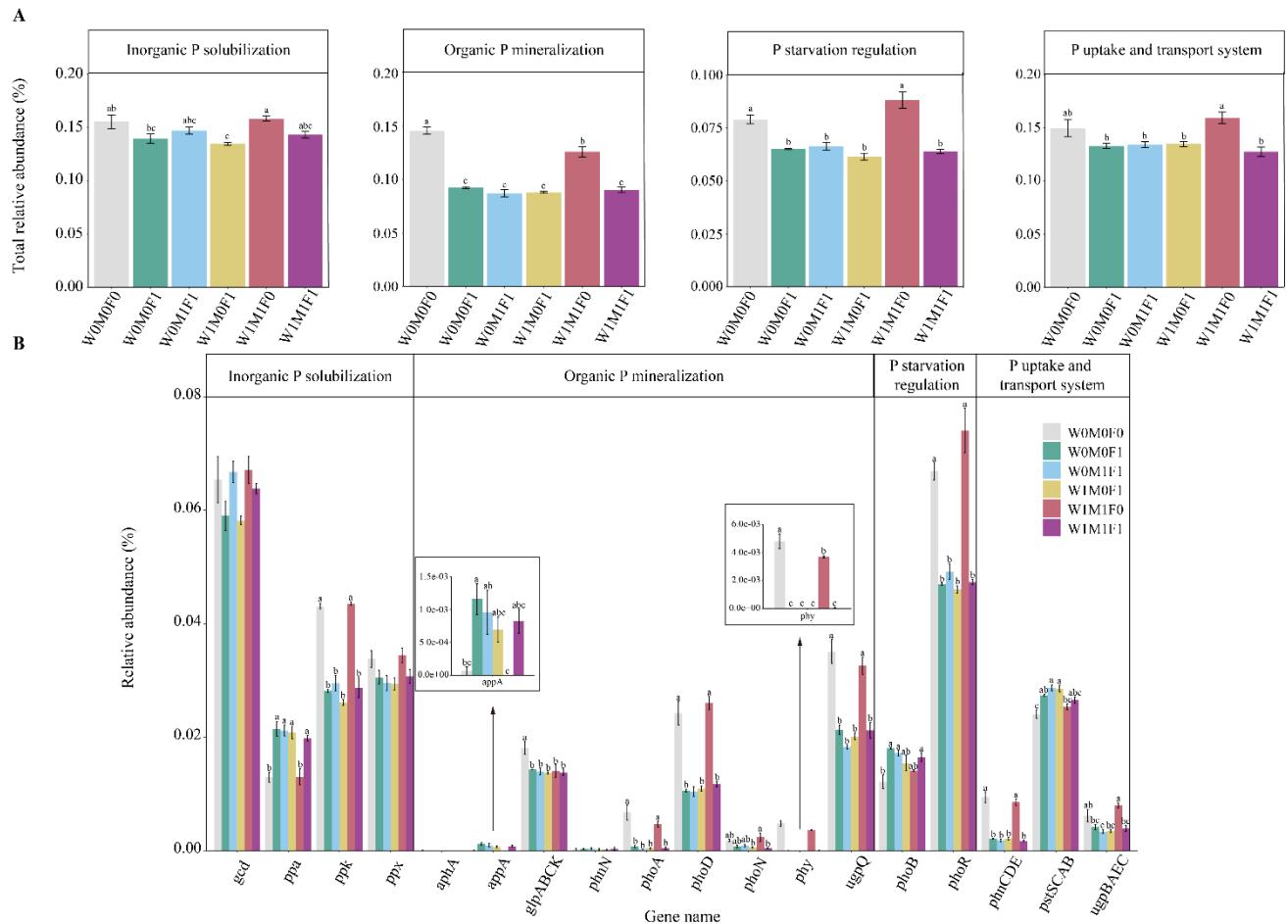


Fig. 2 Relative abundance of genes responsible for microbial inorganic P solubilization, organic P-mineralization, P-starvation regulation, and P-uptake and transport (A) and the individual gene relative abundance (B) in bulk soil

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 upp transporter systems was calculated as the average abundances of gene *uppB*, *uppA*, *uppE*, and *uppC*.

Comment # 21:

Fig. 4: It is confusing. You tried to compare the two fractions, but it is hard to read the information and make the reader easily think you were comparing the six treatments... and lack of description of A and B. I suggest remaking the figure

Response: OK. We have remade the Fig.4 and added the description of A and B.

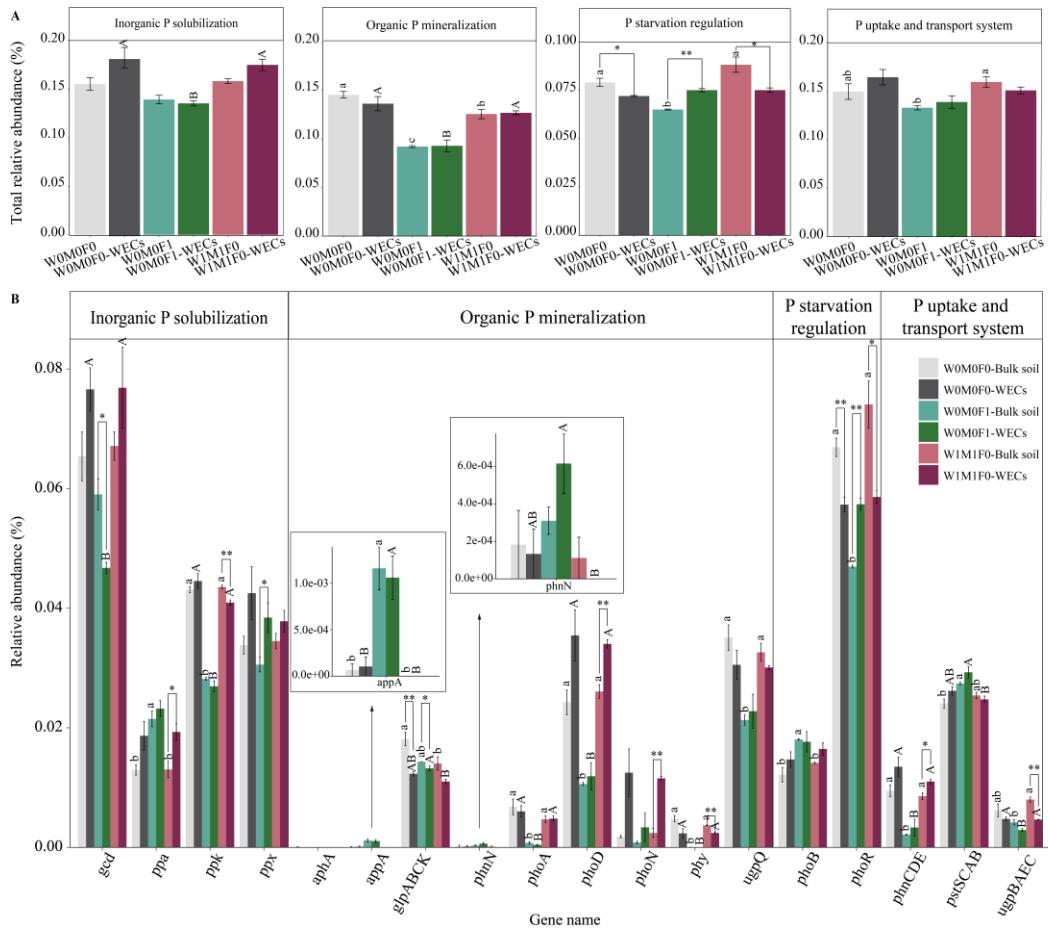


Fig. 4 Relative abundance of genes responsible for microbial inorganic P solubilization, organic P-mineralization, P-starvation regulation, and P-uptake and transport (A) and the individual gene relative abundance (B) in bulk soil and water-extractable colloids (WECs) among the W0M0F0, W0M0F1, and W1M1F0 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 ($< 2\mu\text{m}$) 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.

Comment # 22:

Fig. 5: See comments above... Again, you should always compare them with the control, though you want to compare between two fractions...

Response: Thanks for your suggestion.

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, sole straw retention significantly increased the abundance of the *phoD* gene, whereas mineral fertilization significantly decreased the abundance of the *gcd* gene in WECs compared with bulk soils. Thus, we further performed the taxonomic assignments of *phoD* genes for the W1M1F0 treatment and *gcd* genes for the W0M0F1 treatment between bulk soils and WECs.

The fig.5 highlighted the differences in taxonomic assignments of *gcd* gene for the W1M1F0 treatment and *phoD* gene for the W0M0F1 treatment between bulk soils and WECs.

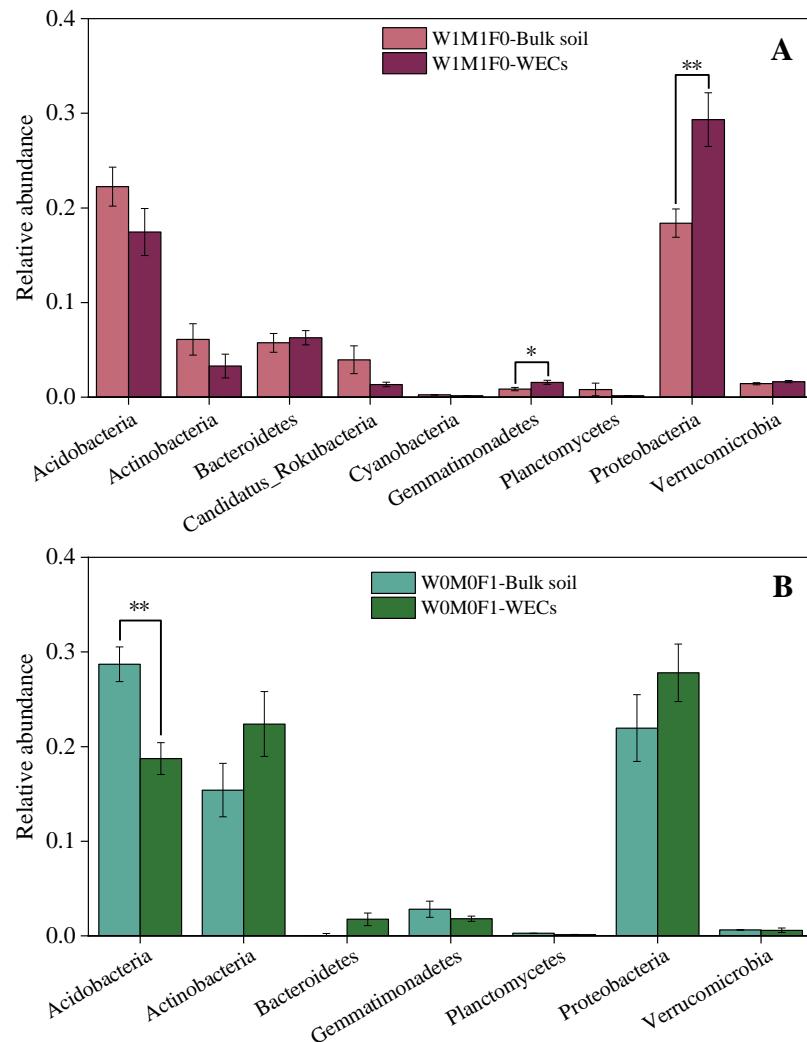


Fig. 5 Taxonomic assignments at the phylum level of the *phoD* gene for the W1M1F0 treatment (A), and the *gcd* gene for the W0M0F1 treatment (B) in bulk soil and water-extractable colloids (WECs)

Comment # 23:

Fig. 6 See comments above... check degree of freedom

Response: The degree of freedom (df) was 5.

The Chi-square/df was typically used to characterize good model fit. We have added it in Line 323-325: The Chi-square/df was 1.8, which was less than 2 and indicated that the SEM model was a superior fit (Alavi et al., 2020).

Comment # 24:

Table 3: There are only 3 treatments in the table

Response: OK, we have modified the Table 3 as suggested.

Table 3 Phosphorus K-edge XANES fitting results (%) showing the relative percent of each P species in water-extractable colloids (WECs) among the W0M0F1, W1M1F0 and W0M0F0 treatments

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 three treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), and (3) both wheat and maize straw retention with no fertilizer (W1M1F0), respectively. DCP, dibasic calcium phosphate dihydrate (DCP, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$); Al-P, aluminum phosphate (AlPO_4); Fe-P, iron phosphate dihydrate ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$); and IHP, inositol hexakisphosphate. Values in each column followed by the different lowercase letters indicate significant differences ($P < 0.05$).

Comment # 25:

Table 5: There are only 3 treatments in the table

Response: *OK, we have modified the Table 5 as suggested.*

Table 5 Concentrations (mg/kg) of P species in water-extractable colloids (WECs) evaluated in the solution ^{31}P NMR analysis among the W0M0F1, W1M1F0 and W0M0F0 treatments

Samples	Inorganic P			Organic P						
	NaOH-Na ₂ EDTA extracted 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 three treatments were: (1) the control treatment, without straw retention and mineral fertilizer (W0M0F0), (2) single application of mineral fertilizer (W0M0F1), and (3) both wheat and maize straw retention with no fertilizer (W1M1F0) 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 ($P < 0.05$).

Comment # 26:

Discussion

The subtitles should be informative and clear, with bullet points emphasized. In addition, all the figures and tables described and shown in the Result section need to be used and properly referenced in the Discussion section; otherwise, the results do not need to be in the Result section. Cite only the necessary literature.

Response: *Thanks for your suggestion.*

The subtitles were revised as suggested:

4.1 Mineral fertilization restricted genes involved in P transformation in bulk soils

4.2 Straw retention increased the abundances of *phoD* gene and *phoD*-harbouring *Proteobacteria* in WECs

The figures and tables were properly referenced in the Discussion section. The necessary literature was cited.

Comment # 27:

Line 326- 'to the enhanced organic matter from crops...' see comments above, how did you harvest the crops with

sole mineral fertilizer input?

Response: Thanks for your suggestion. For the sole mineral fertilizer treatment, straws were removed and the roots were left in the field.

We have added it in Line 112-113 of the Materials and methods section.

Line 112-113: For the W0M0F1 treatments, straws were removed and the roots were left in the field.

Comment # 28:

Line 342: 'Consistent with our findings...' Normally, it should be 'consistent with previous studies, our study finds...'. The discussion section is to interpret your data and deliver your findings/opinions to the audience instead of describing other studies.

Response: We have revised it as follows:

Line 350-353: Consistent with previous findings (Ikoyi et al., 2018; Dai et al., 2020), mineral fertilization alone or combined with straw retention 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).

Comment # 29:

Line 344: 'Long-term P...' is this also mineral P fertilizer?

Response: Yes. 'Long-term P...' is also mineral P fertilizer in the paper (Dai et al., 2020).

Comment # 30:

Line 346-353: Where is your own data? Please reference it and demonstrate it.

Response: OK, we have revised as suggested.

Line 354-364: Additionally, Chen et al. (2017) identified soil pH as the primary factor influencing the compositions of microbial community harboring the *phoD* gene, noting a positive correlation between soil pH and of the *phoD* gene abundance. 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. In this study, Spearman's Rank correlations showed 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 P-cycling related genes (Fig. 2) can be attributed to increased soil P contents and low soil pH (Table 1 and 4) under mineral fertilization compared with the control treatment.

Comment # 31:

Line 359: I assume you tried to compare with the control treatment?

Response: Yes. We have added it.

Line 362-364: Thus, the decline in the abundance of P-cycling related genes (Fig. 2) can be attributed to increased soil P contents and low soil pH (Table 1 and 4) under mineral fertilization compared with the control treatment.

Comment # 32:

Line 365: If you want to discuss this, then you need to describe it first the CN or CP ratio first in the Result section and reference it in the Discussion section accordingly.

Response: Thanks for your suggestion.

The C/N in wheat and maize straw was not measured and it was in the range of 52-73:1 according the previous studies. This information was provided in Line 369-371: 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.

Comment # 33:

Line 368: Concluded from Table 1?

Response: Yes. We have added it.

Line 372-373: Thus, the straw retention for 13 years did not show any significant impact on soil C, N, P nutrients (Table 1).

Comment # 34:

Line 371: 'The slight increase ...' confused

Response: Thanks for your suggestions. We have revised it.

Line 377-378: The increase in MBC resulted in the increase of MBP (Spohn and Kuzyakov, 2013), as shown in Table 1.

Comment # 35:

Line 372: Combine your own data

Response: Thanks for your suggestion. We have added 'as shown in Table 1' in Line 377-378.

Line 377-378: The increase in MBC resulted in the increase of MBP (Spohn and Kuzyakov, 2013), as shown in Table 1.

Comment # 36:

Line 376: 'Had remarkable influences on...' make it clear, the influence is increase or decrease?

Response: Thanks for your suggestion. We have revised it as suggested.

Line 381-383: In this study, straw retention incorporated with mineral fertilization brought remarkable decreases in soil pH and significant increases in soil nutrients, which was significantly different from sole straw retention.

Comment # 37:

Line 378-379: It is better to have a solid summary based on your data and findings. This finding is not new as the OM decomposition will release organic acid. I expect to see more interesting results from your data.

Response: We have revised it as suggested.

Line 384-386: Sole straw retention showed minimal effects on soil properties, P species and transformation genes in bulk soil. Interestingly, it has started to have a notable influence on these indicators in the soil colloids (WECs), as discussed below.

Comment # 38:

Line 381: 'AP'?

Response: AP is the available P. The abbreviation of AP was described in Line 150-151: Available P (AP, Olsen-P) concentration was quantified by Olsen and Sommers (1982).

Comment # 39:

Line 383: 'The influences of...' Make your opinions clear with referenced results; how do you define the stronger effect?

Response: We have revised the description as suggested.

Line 389-396: The higher concentrations of SOC, TN, TP, AP and various P species in WECs (Fig. 1 and Table 5) compared with bulk soil (Table 1 and 4) indicated that nutrients are enriched within the WECs due to their high specific surface area (Jiang et al., 2014). Mineral fertilization and straw retention caused significant increases in these indicators within the WECs compared to bulk soil, suggesting that the management practices exerted more significant impacts on soil properties and P species within the WECs when compared to the effects observed in bulk soils. This highlighted the heightened sensitivity of the physicochemical properties of soil microparticles to environmental disturbances compared to bulk soil.

Comment # 40:

Line 394: 'slight increase' is it significant?

Response: The slight increase meant the concentrations of TP and each P species for WECs increased, while there was no statistics significant difference between WECs and bulk soil.

Comment # 41:

Line 395: 'considerable influence...' How much is the influence? increase or decrease?

Response: Thank you for pointing that out. We have revised it as suggested.

Line 404-406: This indicated that straw retention promoted the accumulation of nutrients on WECs, which could enhance the supply and cycling of P.

Comment # 42:

Line 399: 'Research conducted...' It seems these are not related to your context.

Response: Thank you for pointing that out. We have modified the citation and revised it as suggested.

Line 407-420: 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. 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). Research conducted by Fierer et al. (2012) and Ling et al. (2014) have shown that higher concentrations of total N, P and organic C could promote the growth of such microorganisms. In our research, the notable increases in SOC, TN and each P specie in WECs under straw retention likely created favorable conditions for the proliferation of copiotrophic bacteria (e.g., Proteobacteria).

Comment # 43:

Line 410: 'clay particles'? <2 micrometers will be enough, in my opinion.

Response: We have revised it as suggested.

Line 420-421: Generally, the WECs (clay particles) including natural organic matter (e.g., humus) and

inorganic colloids (silicate, and Al/Fe oxides)

Comment # 44:

Line 419: Check the logic framework and how you get to this conclusion.

Response: *OK. We have revised it as suggested.*

*Line 407-430: 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. 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). Research conducted by Fierer et al. (2012) and Ling et al. (2014) have shown that higher concentrations of total N, P and organic C could promote the growth of such microorganisms. In our research, the notable increases in SOC, TN and each P specie in WECs under straw retention likely created favorable conditions for the proliferation of copiotrophic bacteria (e.g., Proteobacteria). Generally, the WECs 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 μ m soil particulates (Oliver et al., 2007). The population of the bacteria (*Pseudomonas putida*) attached to the clay particle in Red soil (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 by itself characteristics and the increased the nutrients contents of WECs under straw retention.*

Comment # 45:

Line 422: Check the logic framework and how you get to this conclusion.

Response: *We have revised it as suggested.*

Line 431-436: In this study, mineral fertilization also caused the enhancements of SOC contents in WECs (Fig. 1), which positively influenced the abundance of P cycling genes. However, it was also noted that mineral fertilization led to a dramatic increase in P contents and a substantial decrease in soil pH by 1.76-1.89 units (Table 1), which restricted the expression and activity 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.

References

Alavi M, Visentin D C, Thapa D K, Hunt G E, Watson R, Cleary M. 2020. Chi-square for model fit in confirmatory factor analysis. *Journal of Advanced Nursing*. **76**: 2209-2211.

Chen X D, Jiang N, Chen Z H, Tian J H, Sun N, Xu M G, Chen L J. 2017. Response of soil phoD phosphatase gene to long-term combined applications of chemical fertilizers and organic materials. *Applied Soil Ecology*. **119**:

197-204.

Dai Z M, Liu G F, Chen H H, Chen C R, Wang J K, Ai S Y, Wei D, Li D M, Ma B, Tang C X, Brookes P C, Xu J M. 2020. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *The ISME Journal*. **14**: 757-770.

Ikoyi I, Fowler A, Schmalenberger A. 2018. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. *Science of The Total Environment*. **630**: 849-858.

Jiang C L, Séquaris J-M, Wacha A, Bóta A, Vereecken H, Klumpp E. 2014. Effect of metal oxide on surface area and pore size of water-dispersible colloids from three german silt loam topsoils. *Geoderma*. **235-236**: 260-270.

Li C Y, Hao Y h, Xue Y L, Wang Y, Dang T H. 2020. Effects of long-term fertilization on soil microbial biomass carbon, nitrogen, and phosphorus in the farmland of the loess plateau, china. *Journal of Agro-Environment Science*. **39**: 1783-1791.

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

Rousk J, Bååth E, Brookes P C, Lauber C L, Lozupone C, Caporaso J G, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a ph gradient in an arable soil. *The ISME Journal*. **4**: 1340-1351.

Schumacher B. 2002. Methods for the determination of total organic carbon (toc) in soils and sediments. *Ecological Risk Assessment Support Center Office of Research and Development*.

Wu L, Zhang W J, Wei W J, He Z L, Kuzyakov Y, Bol R, Hu R G. 2019. Soil organic matter priming and carbon balance after straw addition is regulated by long-term fertilization. *Soil Biology and Biochemistry*. **135**: 383-391.