

## Cover letter

Dear editors,

Thank you for your letter and the constructive suggestions from the reviewers regarding our manuscript. These comments are invaluable and will greatly assist us in revising and improving our paper. In light of the suggestions and the reviewers' comments, we have revised the paper entitled "*Effect of straw retention and mineral fertilization on P speciation and P-transformation microorganisms in water extractable colloids of a Vertisol*" (Manuscript number: *EGUSPHERE-2024-983*). The revised portions are marked in track change in the manuscript. Appended to this letter is our point-by-point response to the comments raised by the reviewers. If you have any questions regarding this paper, please do not hesitate to contact us.

We also would like to thank you for allowing us to resubmit a revised copy of the manuscript and hope that the revised manuscript will be acceptable for publication in *Biogeosciences*.

Looking forward to hearing from you as soon as possible.

Your Sincerely,

Xiaoqian Jiang

## Response to Reviewer Comments

### Responses to Reviewer 1:

thank you for considering and following the reviewer's suggestions, in particular to improve the Figures. However, I would request some further changes, and as also already previously pointed out by the reviewers earlier, I would recommend to have some quick language check.

Moreover, please also add some more details in the methods sections (some details are specified below in the line-by-line section). Please pay particular attention especially on the section 2.6 DNA extraction and metagenomics analysis. The details provided are still quite vague in some sections and it would be important to add more technical details. Please mention how the reads were filtered, and provide details on the pre-processing steps and a brief statement with details on the Diamond settings that have been used.

Finally, I would highly encourage to restructure the conclusions sections. At the moment the mostly summarize the results, only the very last sentence is rather a conclusion. To make the manuscript stronger, I would recommend that the authors could rephrase and rather focus on impacts or implications of the outcomes of their study. Below are some points I would like you to resolve specifically.

***Response:*** *Thank you very much for your careful review and valuable suggestions. We highly appreciate your efforts in helping us improve our manuscript.*

*Regarding your request for further changes to improve the figures, we have improved the figures and enhance them as per your instructions.*

*As for the language check, we have conducted a thorough review and correction to ensure the language is accurate and fluent.*

*In the methods section, we have added more details as you have requested.*

*We have rephrased the conclusions and focus on the impacts and implications of our study outcomes.*

### ***Conclusion :***

This study provides valuable insights into P speciation and the role of P transformation microorganisms at the soil microparticle scale (WECs) in the context of straw retention and mineral fertilization. Our findings underscore the critical influence of these management practices on soil chemistry and microbial dynamics. The decrease in soil pH and increases in soil TP under mineral fertilization hinder the expression of genes related to P transformation in bulk soils, potentially limiting the efficiency of P cycling. In contrast, straw retention enhances the accumulation of organic C and total N in soil colloids scale significantly, thus causing significant increase in the abundance of gene encoding for alkaline phosphatase (*phoD*) and *phoD*-harbouring *Proteobacteria* for WECs. It indicates that straw retention could potentially improve P availability by increasing P mineralization capacity of WECs. This information provided innovative evidence 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.

### **Comment # 1:**

Line 26: remove the last 'in bulk soil'.

***Response:*** *Thank you for your suggestion. We have removed “in bulk soil ” in Line26.*

### **Comment # 2:**

Line 28: change to: ', but lead to increases in..'

**Response:** Thank you for your suggestion. We have revised it as suggested.

**Comment # 3:**

Line 29: this sentence is not really easy to understand, which treatment caused higher P cycling gene abundance compared to which?

**Response:**

*We appreciate your insights, and we have revised the sentence.*

*Line 29-31: Furthermore, compared with mineral fertilization, straw retention caused significantly greater differences in the relative abundances of P cycling genes between WECs and bulk soils..*

**Comment # 4:**

Line 47: when you use the Ca-P, Al-P etc... please first time spell them out or explain a bit better.

**Response:** We have revised the manuscript to spell out and provide a brief explanation of the terms *Ca<sub>2</sub>-P*, *Al-P*, *Fe-P* and *Ca<sub>10</sub>-P*.

*Line 46-49: Under mineral fertilization and straw retention, dicalcium phosphate (Ca<sub>2</sub>-P), iron-bound P (Fe-P) and aluminum-bound P (Al-P) contents increased, but apatite (Ca<sub>10</sub>-P) concentration reduced, thereby promoting the transformation of P fractions (Xu et al., 2022).*

**Comment # 5:**

Line 63: delete the space before 0.25

**Response:** Thank you for pointing that out. We have deleted the space before 0.25.

**Comment # 6:**

Line 91: this first sentence is a bit out of context here. Maybe change to:

In a long-term (13 year) field experiment modulating straw retention and mineral fertilization, we investigated 1) the responses....

**Response:** We have revised the sentence as suggested.

**Comment # 7:**

Line 95: These sentences could be removed, as they are almost repeating the same message as just before the research aim (Lines 89-90)

**Response:** Thank you for pointing that out. We have removed the sentence.

**Comment # 8:**

Line 124: Change to: From all six treatment plots soil samples were collected after wheat harvest in June 2021

**Response:** OK. We have modified the description in Line 124.

*Line 124: From all six treatment plots soil samples were collected after wheat harvest in June 2021.*

**Comment # 9:**

Line 128: delete 'the' before 'acid and alkaline phosphatase'

**Response:** OK. We have deleted 'the' in Line 128.

*Line 126-128: The first subsample was preserved at 4 °C to examine soil microbial biomass C (MBC) and microbial biomass P (MBP), along with acid and alkaline phosphatase activities (ACP and ALP).*

**Comment # 10:**

Line 151: please add here, to remove inorganic carbon (and change stop to stopped).

**Response:** *Thank you for your suggestion. We have revised the sentence as suggested.*

**Line 149-150:** *For SOC measurement, 1M HCl was added to the samples in small increments until effervescence stopped to remove inorganic carbon (Schumacher, 2002).*

**Comment # 11:**

Line 154: change to 'using the method described by Olsen...'

**Response:** *Thank you for your suggestion. We have revised the sentence.*

**Line 152-153:** Available P (AP, Olsen-P) concentration was quantified using the method described by Olsen and Sommers (1982).

**Comment # 12:**

Line 155: leave the abbreviation in brackets: dissolved organic carbon (DOC).

**Response:** *Thank you for your feedback. We have added the abbreviation in brackets.*

**Line 156-157:** The dissolved organic C (DOC) was quantified as the extracted organic C by K<sub>2</sub>SO<sub>4</sub> from the non-fumigated samples (Wu et al., 2019).

**Comment # 13:**

Line 160 and 162: provide references for the conversion factor

**Response:** *Thank you for pointing that out. We have provided references for the conversion factor.*

**Line 158-161:** 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 (Vance et al., 1987). MBP was calculated as the variation in extractable P with 0.5 M NaHCO<sub>3</sub> between the non-fumigated and fumigated soil samples, with a conversion factor of 0.40 (Brookes et al., 1982).

**Comment # 14:**

Line 200: change to: DNA was extracted using a Fast...

**Response:** *Thank you for pointing that out. We have revised the sentence.*

**Line 199:** *Soil DNA was extracted using a FastDNA Spin kit (MP Biomedicals, USA).*

**Comment # 15:**

Line 233: change 'agricultural managements' to 'agricultural management types'

**Response:** *Thank you for your suggestion. We have revised the sentence.*

**Line 233-235:** *Structural equation modeling (SEM) was used to explore the relationships among agricultural management types, soil properties, and P-cycling related genes by Amos (24.0).*

**Comment # 16:**

Line 238: change 'incorporated' to 'in combination with'

**Response:** *Thank you for your suggestion. We have revised it as suggested.*

**Line 239:** *Straw retention in combination with mineral fertilization (i.e., W0M1F1, W1M0F1, W1M1F1) decreased soil pH from 6.90 to 5.01.*

**Comment # 17:**

Line 239: better mention the values changed from pH x to y than the differences

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

**Line 239:** *Straw retention in combination with mineral fertilization (i.e., W0M1F1, W1M0F1, W1M1F1) decreased soil pH from 6.90 to 5.01.*

**Comment # 18:**

Line 240 and throughout the manuscript: use SI-units e.g. g kg<sup>-1</sup> or µg g<sup>-1</sup> h<sup>-1</sup>

**Response:** *Thank you for your feedback. We have revised the units throughout the manuscript.*

**Comment # 19:**

Line 243: change to: there was not significant effect of straw retention detectable

**Response:** *Thank you for your suggestion. We have revised it as suggested.*

**Line 246-248:** *There was not significant effect of sole straw retention (i.e., W1M1F0) detectable except for slight increases in soil MBC and MBP contents compared with the control treatment (Table 1).*

**Comment # 20:**

Line 247-250: this is very vague, can this turned more specific maybe?

**Response:** *Thank you for pointing that out. We have revised the descriptions.*

**Line 249-251:** *Mineral fertilization indeed enhanced soil nutrient contents. It also led to soil acidification, which was not effectively alleviated by the return of straw in combination with mineral fertilization.*

**Comment # 21:**

Line 279: remove 'that' after manifested

**Response:** *Thank you for your suggestion. We have removed 'that' after manifested in Line 283.*

**Line 282-283:** *These results manifested the effect of mineral fertilization on P species concentration was more apparent than that of straw retention.*

**Comment # 22:**

Line 283: remove 'the' before samples

**Response:** *Thank you for your suggestion. We have removed 'the' before samples in Line 287.*

**Line 284-286:** *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 tested samples (Tables 4 and 5).*

**Comment # 23:**

Line 286: stick to uniform tenses

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

**Line 287-290:** *Relative to the control, the concentrations of orthophosphate, orthophosphate monoesters and orthophosphate diesters rose sharply after mineral fertilization for WECs, while the significant increase of only orthophosphate concentrations was detected for bulk soils.*

**Comment # 24:**

Line 316: remove 'the' before treatments

**Response:** *Thank you for your feedback. We have removed 'the' before treatments in Line 320.*

**Line 318-319:** *Additionally, the bacterial  $\beta$ -diversity in WECs showed a clear divergence from those in bulk soils for all treatments (Fig. S7).*

**Comment # 25:**

Line 322: this is not a full sentence, please modify (whereas at the begin of the sentence needs to be changed).

**Response:** *Thank you for your suggestions. We have revised it as suggested.*

**Line 323-325:** *Specially, a strong correlation was detected between the majority of P cycling genes and soil nutrients including C, N, P in bulk soils. In contrast, no consistent trends were observed in WECs.*

**Comment # 26:**

Line 390: ... I suggest to change to: could be caused by the higher specific surface area of the WEC

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

**Line 392-394:** *The higher concentrations of SOC, TN, TP, AP and various P species in WECs (Fig. 1 and Table 5) compared with bulk soil (Tables 1 and 4) indicated nutrient enrichment within the WECs. This could be caused by the higher specific surface area of the WECs (Jiang et al., 2014).*

**Comment # 27:**

Line 391 and general, some sentences are very long and could be shortened

**Response:** *Thank you for your feedback. We have revised the sentences.*

**Line 394-396:** *Significant increases in these indicators suggested that the management practices exerted more substantial impacts on soil properties and P species in WECs than in bulk soils.*