

Response to Anonymous Referee #1 (RC1):

Comment: The manuscript idea is wonderful and interesting, as the study aims to evaluate the effect of different ratios of straw to biochar (incubation experiment for 90 day) to provide an optimal strategy for treating soil contaminated with cadmium and zinc, and to study their ability to treat soil contaminated with these heavy metals, and also to study the availability of microbial nutrients, the carbon cycle, and the elements available in the soil (soil fertility). The topic is important, and manuscript fits with the scope of the journal. The manuscript is very well written, the effort is very evident, and the way the results are presented is excellent. But it has some weaknesses associated with the presented data and discussion have shortcomings as discussed below. Therefore, the current version of the manuscript needs major revision to be published in (egusphere). There are several issues must be addressed.

Response: Thank you for your valuable comment. Your suggestion is constructive on the improvement of this manuscript. We have made revisions to the manuscript according to your suggestion. The specific revisions are as follows.

Comment: 1. Line 64: What is the texture of the soil used in the study?

Response: OK, the experimental soil is loamy tidal soil. This information was added.

Manuscript text: The experimental soil is loamy tidal soil. (Line 67)

Comment: 2. Line 96: please add the reference of microbial biomass estimation.

Response: OK! The reference of microbial biomass estimation was provided.

Manuscript text: Microbial biomass was assessed using soil samples collected on day 90. The chloroform fumigation method was used to determine microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP), calculated from the difference between the fumigated and non-fumigated samples (Hu et al., 2025a). (Line 101-103)

Hu. J., Cui. H., Fan. M., Liu. M., Wang. S., Li. X., Peng. X., Shi. F., Song. W., and Sun W.: Functional soil microbes drive soil phosphorus fractions in response to nitrogen addition across aggregate levels, *Front. Microbiol.*, 16, 1671863. <https://doi.org/10.3389/fmicb.2025.1671863>, 2025a. (Line 429-431)

Comment: 3. Line 110: in statical analysis, the experiment was CRD or WHAT....?

Response: Yes, the experiment was designed by completely randomized design (CRD), which was provided.

Manuscript text: The incubation experiment adopted the completely randomized design (CRD), with 9 replicates set for each treatment. (Line 77-78)

Comment: 4. Line 119: It says the results were on days 30, 60, and 90... Please clarify this in the materials section.

Response: We have revised the description of sampling time in the Materials and Methods section. Destructive sampling was conducted on days 30, 60 and 90 for each treatment. Most indicators are measured at these three time points. The measurement time of special indicators (Organic C fractions, CO₂ emission and microbial biomass) has been clarified.

Manuscript text: Destructive sampling was conducted on days 30, 60 and 90 for each treatment. (Line 81-82)

The alkali absorption method (Geng et al., 2022) was used to measure the soil CO₂ emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90. (Line 97-98)

Microbial biomass was assessed using soil samples collected on day 90. (Line 101)

Comment: 5. In Results and discussion section.... Please write the value of heavy metals concentration not the reduction rate only to clarify the result more.

Response: Thanks for your suggestion. The value of heavy metals concentration was written. And, we have appropriately supplemented the measured values in the results analysis section.

Manuscript text: Soil DTPA-extractable Cd and Zn were measured to evaluate the effects of straw and biochar additions (Fig. 1). DTPA-extractable Cd content on days 30, 60 and 90 of CK was 0.220-0.230 mg kg⁻¹ (Fig. 1a), and for S3C1, S2C2, S1C3 and S0C4 treatments, the DTPA-extractable Cd content were 0.197–0.143 mg kg⁻¹ (Day 30), 0.193–0.139 mg kg⁻¹ (Day 60), and 0.218–0.143 mg kg⁻¹ (Day 90), which were reduced by 10.60–35.34% (Day 30), 12.30–37.04% (Day 60), and 10.88–37.73% (Day 90), compared to CK. Meanwhile, the DTPA-extractable Zn content of CK on days 30, 60 and 90 were 122.04 mg kg⁻¹, 121.62 mg kg⁻¹, and 120.66 mg kg⁻¹, respectively (Fig. 1b). (Line 125-130)

By day 90, cumulative CO₂ emissions of CK and S0C4 were 1215.28 mg kg⁻¹ and 1391.81 mg kg⁻¹, respectively. (Line 148-149)

On days 30, 60 and 90, the SOC content of CK treatment was 10.47 g kg⁻¹, 10.47 g kg⁻¹ and 10.39 g kg⁻¹ (Fig. S1). (Line 173-174)

On day 90, AHN content in S2C2, S1C3 and S0C4 was 71.81 mg kg⁻¹, 77.90 mg kg⁻¹ and 74.16 mg kg⁻¹, respectively, which was 14.79%, 24.52% and 18.53% higher, than that in S4C0. (Line 208-209)

The MBC content of S1C3 was significantly higher than that of S4C0, S3C1, S2C2 and S0C4. Compared with S4C0, soil MBN in S3C1, S2C2, S1C3 and S0C4, increased by 18.87–31.32%. The soil MBP content of S1C3 differed significantly from that of S4C0. Overall, the MBC, MBN and MBP contents treated with S1C3 were 283.44 mg kg⁻¹, 44.02 mg kg⁻¹ and 16.51 mg kg⁻¹, which were the highest levels, and S1C3 is the best treatment to increase soil microbial biomass. (Line 229-232)

For S4C0, the β-glucosidase, urease and alkaline phosphatase activities on day 30 were 98.44 nmol·g⁻¹·h⁻¹, 69.19 nmol·g⁻¹·h⁻¹ and 61.42 nmol·g⁻¹·h⁻¹, respectively, with the highest levels at the three sampling

times. (Line 262-264)

Comment: 6. Line 145: please add reference.

Response: OK! The reference was added.

Manuscript text: By day 90, cumulative CO₂ release in S0C4 was over 20% higher than that in CK (Fig. 2b), likely owing to a priming effect stimulating mineralization of native soil organic matter (Dong et al., 2026). (Line 156-157)

Dong, H., Fu, Y., Dai, S., He, P., Luo, Y., Oliver, M. A., Horwath, W. R., and Li, L. J.: Priming effects on soil organic matter mineralization by carbon substrates: A global meta-analysis, *Global Change Biology*, 32, e70861. <https://doi.org/10.1111/gcb.70861>, 2026. (Line 399-401)

Comment: 7. Figure 2: illustrate CO₂ flux and (b) accumulation of different treatments in different time.... please clarify it in material section.

Response: OK! The measurement times of CO₂ flux and accumulation was added.

Manuscript text: The alkali absorption method (Geng et al., 2022) was used to measure the soil CO₂ emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90. (Line 97-98)

Comment: 8. In general, please specify the days on which the measurements were taken in more detail in material section.

Response: OK! We have revised the description of sampling time in the Materials and Methods section. Destructive sampling was conducted on days 30, 60 and 90 for each treatment. Most indicators are measured at these three time points. The measurement time of special indicators (Organic C fractions, CO₂ emission and microbial biomass) has been clarified.

Manuscript text:

We have revised the description of sampling time in the Materials and Methods section. Destructive sampling was conducted on days 30, 60 and 90 for each treatment. Most indicators are measured at these three time points. The measurement time of special indicators (Organic C fractions, CO₂ emission and microbial biomass) has been clarified.

Manuscript text: Destructive sampling was conducted on days 30, 60 and 90 for each treatment. (Line 81-82)

The alkali absorption method (Geng et al., 2022) was used to measure the soil CO₂ emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90. (Line 97-98)

Microbial biomass was assessed using soil samples collected on day 90. (Line 101)

Comment: 9. Please provide a more detailed and clearer explanation of why these transactions were the best or worst in value. What is the underlying reason for this?

Response: OK! We have added the possible reasons for the best comprehensive evaluation of S1C3. Please see Section 3.8.

Manuscript text: 3.8 Comprehensive evaluation

After standardizing the key indices, PCA was performed to comprehensively estimate the improvement effects of different treatments on Cd- and Zn-contaminated soil. The cumulative variance contribution of PC1 and PC2 was 90.901% (Table S5). Based on the PCA coefficient matrix of each index, fuzzy membership function values and comprehensive evaluation scores were further calculated to quantify the comprehensive remediation efficiency of each treatment (Sun et al., 2022). The results showed that S1C3 achieved the best comprehensive score (1.7513), ranking first among all treatments (Table 1). The optimal performance of S1C3 treatment due to the synergistic balance between straw and biochar, which compensated for the shortcomings when either was applied alone or in an imbalanced ratio. Applying straw and biochar at a 1:3 (straw:biochar) ratio balanced heavy metal remediation, carbon sequestration, nutrient availability, and microbial activity, thereby achieving the optimal comprehensive improvement of soil quality on Cd and Zn contaminated soil. (Line 229-338)

Comment: 10. Figure 3: please write down the day the measurement was taken.

Response: OK! The measurement time was added.

Manuscript text: Figure 3. (a) Dissolved organic carbon (DOC), (b) particulate organic carbon (POC), (c) easily oxidizable carbon (EOC), and (d) mineral-associated organic carbon (MAOC) contents of different treatments on day 90. (Line 198-199)

Comment: 11. The title of Figure 4 needs further clarification and detail, with the full name written next to the abbreviations.

Response: OK! The title of Figure 4 has been revised.

Manuscript text: Figure 4. Three-dimensional excitation-emission-matrix (3D-EEM) spectra of (a) CK (control, no straw and biochar), (b) S4C0 (4.0 g straw), (c) S3C1 (3.0 g straw and 1.0 g biochar), (d) S2C2 (2.0 g straw and 2.0 g biochar), (e) S1C3 (1.0 g straw and 3.0 g biochar) and (f) S0C4 (4.0 g biochar). (Line 201-203)

Comment: 12. Line 193: what do you mean by “cultivation progressed”?

Response: We apologize for the confusion we have caused. What I want to express is: “as the incubation experiment progress”. It has been corrected.

Manuscript text: However, as the incubation experiment progressed, AHN content in straw-treated soil gradually decreased (Table S2). (Line 207-208)

Comment: 13. Figure 5: please write down the day the measurement was taken for further clarification.

Response: OK! The measurement time was provided now.

Manuscript text: Figure 5. (a) Alkali hydrolysable nitrogen (AHN), (b) available phosphorus (Olsen-P), and (c) available potassium (AK) contents of different treatments on days 30, 60 and 90. (Line 202-203)

Comment: 14. Line 208: Soil microbial biomass. Please write the discussion in a more detailed manner and cite scientific references in this section.

Response: OK! We have revised the section on soil microbial biomass. Further discussion was conducted and more relevant references were cited. Please see Section 3.5.

Manuscript text: 3.5 Soil microbial biomass

Soil microbial biomass is a core biological indicator reflecting soil microbial activity and nutrient sequestration (Chen et al., 2025a). Soil microorganisms utilize energy obtained from soil organic matter through respiration to create new biomass, thereby sequestering C and N (Elrys et al., 2025). After completion of the incubation experiment, treatment with straw, biochar or their combination increased soil MBC, MBN and MBP by 24.73–61.41%, 20.77–58.60% and 23.76–47.61%, respectively (Fig. S3). The MBC content of S1C3 was significantly higher than that of S4C0, S3C1, S2C2 and S0C4. Compared with S4C0, soil MBN in S3C1, S2C2, S1C3 and S0C4, increased by 18.87–31.32%. The soil MBP content of S1C3 differed significantly from that of S4C0. Overall, the MBC, MBN and MBP contents treated with S1C3 were 283.44 mg kg⁻¹, 44.02 mg kg⁻¹ and 16.51 mg kg⁻¹, which were the highest levels, and S1C3 is the best treatment to increase soil microbial biomass.

Although biochar improved soil available N, P, and K nutrients at the end of incubation (Fig. 5), however, its C is mainly composed of stable aromatic C structures, which have strong chemical stability and are difficult to be decomposed and utilized by soil microorganisms, thus induced significant limitations in C utilization, (Chen et al., 2024b). When straw combined with biochar, both can alleviate heavy metal stress to enhance soil microbial biomass and provided a sustainable C, N and P nutrients for microbial growth and reproduction (Ninkuu et al., 2025). Thus, combining straw and biochar, especially at the S1C3 ratio, was most effective in promoting microbial growth and increasing soil microbial biomass. (Line 224-238)

Comment: 15. Line 265: Correlation and principal component analysis. Please write the discussion in more detail and cite scientific references in this section.

Response: OK, we have added some discussions and scientific references in the Correlation and Principal Component Analysis section as per your suggestion. Please see Section 3.7.

Manuscript text: 3.7 Correlation and principal component analysis

The correlation between the 17 indices and the amounts of straw and biochar applied was analysed (Fig. 7a). DTPA-Cd showed a significantly positive correlation with DTPA-Zn and a negative correlation with AHN, AK, MAOC, MBN and MBP. This result indicated that the accumulation of bioavailable heavy metals in contaminated soil can have a serious inhibitory effect on various soil ecological processes,

limiting soil nutrient supply capacity, organic carbon stabilization processes, and soil microbial reproduction, exacerbating soil ecological function degradation. Kang et al. (2025) also reported a negative correlation between the availability of heavy metals and that of soil available nutrients after biochar application. Positive correlations were observed between available nutrients, microbial biomass, enzyme activities and active organic carbon components, reflecting a synergistic promotion mechanism among soil carbon sequestration, nutrient cycling and microbial activity (Chen et al., 2025b). Differential regulatory effects were observed between straw and biochar application on soil properties. The amount of straw was positively correlated with CO₂ emissions, EOC, urease and alkaline phosphatase. Straw is rich in labile organic carbon components that can be rapidly decomposed and utilized by soil heterotrophic microorganisms (Fang et al., 2025). Straw addition provided a substantial quantity of active C for soil microorganisms, enhanced soil respiration and increased microbial demand for N and P nutrients. The amount of biochar applied was significantly positively correlated with soil available nutrients, MAOC and MBN, suggesting that biochar improves SOC stability and enhances soil nutrient availability. The porous structure, high specific surface area and rich functional groups of biochar can effectively adsorb soil nutrient ions, reduce nutrient leaching loss, and improve soil nutrient availability and retention capacity (Chen et al., 2024b). Meanwhile, biochar can promote the formation of stable organic carbon fractions, and provide a suitable microhabitat for microbial reproduction, thus increasing microbial biomass (Jiang et al., 2025). Additionally, the amount of biochar applied showed a significantly negative correlation with soil DTPA-Cd and DTPA-Zn contents, indicating that biochar effectively immobilized heavy metals in soil and reduced their toxicity. This phenomenon was attributed to mechanisms such as surface adsorption, ion exchange, precipitation, and complexation, which can convert labile heavy metals into stable components, significantly reducing the bioavailability and toxicity of heavy metals (Kang et al., 2024a).

Principal component analysis (PCA) was employed to analyse differences among treatments and relationships among the 17 indices (Fig. 7b). The contribution rates of PC1 and PC2 were 53.3% and 29.3%, respectively; the cumulative contribution rate reached 82.6%, indicating that PC1 and PC2 could reflect the main variation characteristics of soil physicochemical and microbial properties under different treatments, and the PCA results had high data reliability and explanatory power. The points representing straw and biochar treatments were clearly separated from those of CK. The CK points were distributed on the left side of the plot, whereas the straw and biochar treatment points were on the right, demonstrating that straw, biochar or their combined application effectively altered the properties of Cd- and Zn-contaminated soil. Moreover, the distribution of different straw and biochar treatment points varied significantly, suggested that the type and amount of organic improvement materials are key factors affecting the differences in soil properties caused by pollution. Consistent with the correlation network heatmap, the PCA plot also showed negative correlations between soil available heavy metal contents and AHN, AK, MAOC, MBN and MBP. (Line 293-326)

Comment: 16. References are generally very good, but they need to be expanded and cite recent research

related to the research topic.

Response: Ok, more references have been cited to enrich the manuscript.

The newly cited references are as follows:

An, N., Zhang, L., Schoenau, J. J., Liu, Y., Ren, B., Wu, Z., Han, W., and Han, X.: Seven-year straw and biochar amendments modulate soil pore structure, nutrient availability, and nitrogen partial factor productivity, *Agron. J.*, 117, e70171. <https://doi.org/10.1002/agj2.70171>, 2025.

Chen, Y., Liu, S., Huang, Y., Xiao, Q., Zhao, X., Wu, L., and Zhang, W.: Stimulatory effects of nutrient addition on microbial necromass C formation depend on soil stoichiometry, *Geoderma*, 458, 117323. <https://doi.org/10.1016/j.geoderma.2025.117323>, 2025a.

Chen, Y., Sun, K., Yang, Y., Gao, B., and Zheng, H.: Effects of biochar on the accumulation of necromass-derived carbon, the physical protection and microbial mineralization of soil organic carbon, *Crit. Rev. Environ. Sci. Technol.*, 54, 39–67. <https://doi.org/10.1080/10643389.2023.2221155>, 2024b.

Chen, Y., Wu, S., Hu, C.: The synergistic effects of soil acidification and carbon sequestration technologies: Mechanism analysis, research progress, and application prospects, *Geogr. Res. Bull.*, 4, 56–91. https://doi.org/10.50908/grb.4.0_56. 2025b.

Dong, H., Fu, Y., Dai, S., He, P., Luo, Y., Oliver, M. A., Horwath, W. R., and Li, L. J.: Priming effects on soil organic matter mineralization by carbon substrates: A global meta-analysis, *Glob. Chang. Biol.*, 32, e70861. <https://doi.org/10.1111/gcb.70861>, 2026.

Elrys, A. S., Wen, Y., Feng, D., El-Mekkawy, R. M., Kong, M., Qin, X., Lu, Q., Dan, X., Zhu, Q., Tang, S., Wu, Y., Meng, L., and Zhang, J.: Cadmium inhibits carbon and nitrogen cycling through soil microbial biomass and reduces soil nitrogen availability, *J. Hazard. Mater.*, 489, 137524. <https://doi.org/10.1016/j.jhazmat.2025.137524>, 2025.

Fang, K., Chen, J., Wang, G.: Straw incorporation promotes soil health by modulating stable dissolved organic carbon and microbial community in paddy soil, *J. Soil Sediment.*, 25, 2255–2268. <https://doi.org/10.1007/s11368-025-04073-z>, 2025.

Hu, J., Cui, H., Fan, M., Liu, M., Wang, S., Li, X., Peng, X., Shi, F., Song, W., and Sun, W.: Functional soil microbes drive soil phosphorus fractions in response to nitrogen addition across aggregate levels, *Front. Microbiol.*, 16, 1671863. <https://doi.org/10.3389/fmicb.2025.1671863>, 2025a.

Jiang, H., Wu, J., Guan, J., Zhao, F., Tan, L., and Chen, H.: Biochar is an innovative strategy for reconstructing microbial communities and enhancing nutrient utilization efficiency in acidic red soils, *Front. Microbiol.*, 16, 1622408. <https://doi.org/10.3389/fmicb.2025.1622408>, 2025.

Ninkuu, V., Liu, Z., Qin, A., Xie, Y., Song, X., and Sun, W.: Impact of straw returning on soil ecology and crop yield: A review, *Heliyon*, 11, e41651. <https://doi.org/10.1016/j.heliyon.2025.e41651>, 2025.

Xu, L., Zhou, Y., Miao, C., Chen, H., Zhang, J., Qian, H., Hou, P., Ding, Y., Liu, Z., Li, W., Wang, S., Jiang, Y., and Li, G.: Long-term straw return increases fungal residual contribution to soil microaggregate nitrogen pool: An eco-enzymatic stoichiometric study, *Soil Till. Res.*, 244, 106278. <https://doi.org/10.1016/j.still.2024.106278>, 2024.

Ye, Y., Guo, X., Li, Y., Min, W., and Guo, H.: Biochar and straw amendments drive microbial regulation of phosphorus dynamics in saline-irrigated cotton fields, *Front. Microbiol.*, 4, 1508717. <https://doi.org/10.3389/fmbi.2025.1508717>, 2025.

Zhao, D., Yu, W., Brown, R. W., Liu, Z., Xu, Y., Wang, Z., Li, Z., Xu, Y., Xu, P., Jones, D. L., Wen, Y., and Zhou, S.: Straw incorporation strategies regulate detritusphere organic carbon and nutrient accumulation, *Eur. J. Soil Sci.*, 77, e70297. <https://doi.org/10.1111/ejss.70297>, 2026.

Comment: Overall, the research is very good and the idea is excellent, but please cite more references in the discussion as there are sections without definitive references. Also, please include the original measured value compared to the control or initial value, and not just the percentage increase or decrease. The number itself indicates the actual concentration.

Response: Thank you again for your constructive comments. We have appropriately supplemented the measured values in the results analysis section. And more references were cited.

Response to Abel Veloso (RC2):

Comment: General comments

This paper is well written and deals with a subject that is both scientifically relevant and within the journal's scope. The authors evaluated an extensive list of variables, allowing for a comprehensive treatment of the subject. However, there are some points that, in my opinion, should be improved. The discussion could be strengthened by further comparing the results obtained by the authors with those of other studies. Additionally, some of the conclusions drawn by the authors should be more firmly supported by the results and, in particular, by the statistical analysis. My detailed comments are provided

Response: We sincerely appreciate your valuable time and constructive comments, which effectively improve the integrity and rigor of our manuscript. We have carefully revised the whole manuscript based on all your suggestions, and we list our detailed responses to each comment below.

Comment: Introduction

At high concentrations, Zn can cause several problems, including phytotoxicity. However, it is also a plant micronutrient, which highlights its importance, provided that its soil content remains within reasonable limits. In my opinion, this should be briefly focused.

Response: Thanks for your suggestion. We have provided a brief explanation of the toxic effects of excessive zinc on plants.

Manuscript text: Cd exhibits high biological toxicity and carcinogenic potential, even for excessive Zn, inhibit plant growth by reducing photosynthetic pigment content and affecting enzyme activity, although Zn is an essential trace element for plants (Natasha et al., 2022). (Line 29-31)

Comment: I also think that the possible effects of straw and biochar on soil microorganisms and soil enzymes are not sufficiently addressed in the Introduction.

Response: OK, we have further explained the effects of biochar and straw on soil microorganisms and enzymes in the Introduction.

Manuscript text: Notably, coupled with the heavy metals stress alleviation, soil microbial activity is improved, and increasing their nutrient requirements, however, its stable C structure limits its availability as a microbial carbon source, which may exacerbate nutrient limitations (Chen et al., 2022). (Line 42-45)

Organic matter in straw is more easily decomposed, providing a sustainable source of activated carbon for microorganisms, maintaining stable soil enzyme activity and microbial metabolic functions. However limited studies have investigated the combined influence of straw and remediation agents on soil heavy metals. (Line 52-54)

Comment: Materials and Methods

L63-71 – Some contextualization regarding soil classification, etc. should be given.

Response: The experimental soil is loamy tidal soil. This information was provided.

Manuscript text: The experimental soil is loamy tidal soil. (Line 67)

Comment: Results and Discussion

L127 – p-value should be in lower-case letter.

Response: OK, we have corrected it.

Manuscript text: Corn straw alone also lowered available Cd, reaching significance ($p < 0.05$) on day 60. (Line 137)

Comment: L154 – In my opinion, abbreviations should not be used in titles.

Response: I support your opinion. “SOC” has been changed to full name “Soil organic carbon”. And we have checked and corrected abbreviations in other titles.

Manuscript text: 3.3 Soil organic carbon fractions (Line 166))

Comment: L193-194 – The authors stated that “However, as cultivation progressed, AHN content in straw-treated soil gradually decreased.” This claim should be statistically tested (using an ANOVA and Tukey HSD or Duncan’s post hoc test, or equivalents).

Response: OK. We have supplemented ANOVA and Duncan’s multiple range test to make the analysis more scientifically rigorous.

Manuscript text: However, as the incubation experiment progressed, AHN content in straw-treated soil gradually decreased (Table S2). (Line 207-208)

Table S2. Soil alkaline hydrolysable nitrogen (AHN) content of S4C0 treatment on days 30, 60 and 90.

Treatment	Incubation time	AHN (mg kg ⁻¹)
S4C0	Day 30	85.50±2.56a
S4C0	Day 60	70.05±3.92b
S4C0	Day 90	62.56±3.91c

Comment: L195-196 – This should be supported by reference(s).

Response: A reference that supports our statement was cited.

Manuscript text: This could be attributed to straw decomposition, which releases a large amount of available N (Zhao et al., 2026). (Line 210)

Zhao, D., Yu, W., Brown, R. W., Liu, Z., Xu, Y., Wang, Z., Li, Z., Xu, Y., Xu, P., Jones, D. L., Wen, Y., and Zhou, S.: Straw incorporation strategies regulate detritusphere organic carbon and nutrient

accumulation, Eur. J. Soil Sci., 77, e70297. <https://doi.org/10.1111/ejss.70297>, 2026. (Line 523-525)

Comment: L199-204 – This should be more thoroughly discussed in light of the available literature.

Response: OK. We have conducted a more in-depth discussion on this section based on available literature.

Manuscript text: On day 30, compared with CK, S2C2, S1C3 and S0C4 significantly increased Olsen-P content by 23.63%, 25.90% and 34.95%, respectively (Fig. 5b). All straw and biochar treatments increased Olsen-P content on days 30 and 60, with S1C3 and S0C4 showing greater improvements than other treatments. Soil AK content exhibited a similar trend to Olsen-P (Fig. 5c): S2C2, S1C3 and S0C4 significantly increased AK content on days 30 and 60. By day 90, all straw and biochar treatments increased soil AK content by 8.50–30.94%. These results were consistent with An et al. (2025), who revealed that straw can release P and K, and biochar and straw can serve as P sources, gradually releasing P through microbial activities (Ye et al., 2025). Between S1C3 and S0C4, no significant differences were detected in soil AHN, Olsen-P and AK across all sampling periods. (Line 214-220)

Comment: L209-218 – Please check my comment above (referring to L199-204).

Response: We have conducted extensive and more in-depth discussions on this section based on existing literature.

Manuscript text: 3.5 Soil microbial biomass

Soil microbial biomass is a core biological indicator reflecting soil microbial activity and nutrient sequestration (Chen et al., 2025a). Soil microorganisms utilize energy obtained from soil organic matter through respiration to create new biomass, thereby sequestering C and N (Elrys et al., 2025). After completion of the incubation experiment, treatment with straw, biochar or their combination increased soil MBC, MBN and MBP by 24.73–61.41%, 20.77–58.60% and 23.76–47.61%, respectively (Fig. S3). The MBC content of S1C3 was significantly higher than that of S4C0, S3C1, S2C2 and S0C4. Compared with S4C0, soil MBN in S3C1, S2C2, S1C3 and S0C4, increased by 18.87–31.32%. The soil MBP content of S1C3 differed significantly from that of S4C0. Overall, the MBC, MBN and MBP contents treated with S1C3 were 283.44 mg kg⁻¹, 44.02 mg kg⁻¹ and 16.51 mg kg⁻¹, which were the highest levels, and S1C3 is the best treatment to increase soil microbial biomass.

Although biochar improved soil available N, P, and K nutrients at the end of incubation (Fig. 5), however, its C is mainly composed of stable aromatic C structures, which have strong chemical stability and are difficult to be decomposed and utilized by soil microorganisms, thus induced significant limitations in C utilization, (Chen et al., 2024b). When straw combined with biochar, both can alleviated heavy metal stress to enhance soil microbial biomass and provided a sustainable C, N and P nutrients for microbial growth and reproduction (Ninkuu et al., 2025). Thus, combining straw and biochar, especially at the S1C3 ratio, was most effective in promoting microbial growth and increasing soil microbial biomass. (Line 224-238)

Comment: L233-234 – In my opinion, saying that " S4C0 had the most pronounced impact on enzyme activities because microorganisms are more responsive to straw" may be too strong and definitive.

Response: We have made appropriate modifications to this sentence to make it less definitive.

Manuscript text: S4C0 had the most pronounced impact on enzyme activities which may be due to the easier utilization of nutrients in straw and the more sensitive response of microorganisms to straw compared to biochar. (Line 252-254)

Comment: L244-246 – These claims should be checked with a statistical test.

Response: We have conducted ANOVA and Duncan's multiple range tests on this section and rewritten the analysis.

Manuscript text: The activities of β -glucosidase, urease, and alkaline phosphatase exhibited distinct temporal dynamics between S4C0 and S0C4 across the 90 days incubation period (Table S3). For S4C0, the β -glucosidase, urease and alkaline phosphatase activities on day 30 were $98.44 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, $69.19 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ and $61.42 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, respectively, with the highest levels at the three sampling times. The activities of β -Glucosidase and alkaline phosphatase decreased on days 60 and 90, and the urease activity decreased on day 90. The initial stimulation of all three hydrolases under S4C0 at day 30 can be attributed to the rapid decomposition of straw, which supplied C, N and P substrates to microbial growth. The subsequent gradual decline of enzyme activities from day 30 to day 90 reflected the progressive depletion of degradable straw components. For S0C4, the β -glucosidase activity peaked on day 30 and significantly decreased on days 60 and 90. On day 30, soil urease activity was significantly higher than that of day 60, while alkaline phosphatase activity changed insignificantly at the three sampling times. The consistently low and temporally stable alkaline phosphatase activity of S0C4 stemmed from the biochemical recalcitrance of biochar. Its highly aromatic structure barely releases bioavailable organic nutrients, failing to induce shifts in phosphorus cycling enzyme throughout the incubation period. (Line 261-272)

Table S3. β -Glucosidase, urease and alkaline phosphatase activities of S4C0 and S0C4 treatment on days 30, 60 and 90.

Treatment	Incubation time	β -Glucosidase ($\text{nmol g}^{-1} \text{ h}^{-1}$)	Urease ($\text{nmol g}^{-1} \text{ h}^{-1}$)	Alkaline phosphatase ($\text{nmol g}^{-1} \text{ h}^{-1}$)
S4C0	Day 30	$98.44 \pm 3.19\text{a}$	$69.19 \pm 7.20\text{a}$	$61.42 \pm 5.20\text{a}$
S4C0	Day 60	$65.16 \pm 4.87\text{b}$	$65.35 \pm 2.68\text{a}$	$43.14 \pm 1.10\text{b}$
S4C0	Day 90	$65.42 \pm 3.15\text{b}$	$54.82 \pm 2.87\text{b}$	$47.24 \pm 3.50\text{b}$
S0C4	Day 30	$67.97 \pm 4.72\text{b}$	$39.29 \pm 2.45\text{d}$	$33.94 \pm 3.19\text{c}$
S0C4	Day 60	$56.02 \pm 4.30\text{c}$	$46.68 \pm 2.65\text{c}$	$34.27 \pm 3.05\text{c}$
S0C4	Day 90	$54.37 \pm 3.81\text{c}$	$43.03 \pm 2.60\text{cd}$	$34.23 \pm 2.77\text{c}$

Comment: L245-246 – While these are possible explanations, the authors do not have a definitive proof that they were the only cause.

Response: We have conducted a more in-depth analysis and discussion on this part.

Manuscript text: The activities of β -glucosidase, urease, and alkaline phosphatase exhibited distinct temporal dynamics between S4C0 and S0C4 across the 90 days incubation period (Table S3). For S4C0, the β -glucosidase, urease and alkaline phosphatase activities on day 30 were $98.44 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, $69.19 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ and $61.42 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, respectively, with the highest levels at the three sampling times. The activities of β -Glucosidase and alkaline phosphatase decreased on days 60 and 90, and the urease activity decreased on day 90. The initial stimulation of all three hydrolases under S4C0 at day 30 can be attributed to the rapid decomposition of straw, which supplied C, N and P substrates to microbial growth. The subsequent gradual decline of enzyme activities from day 30 to day 90 reflected the progressive depletion of degradable straw components. For S0C4, the β -glucosidase activity peaked on day 30 and significantly decreased on days 60 and 90. On day 30, soil urease activity was significantly higher than that of day 60, while alkaline phosphatase activity changed insignificantly at the three sampling times. The consistently low and temporally stable alkaline phosphatase activity of S0C4 stemmed from the biochemical recalcitrance of biochar. Its highly aromatic structure barely releases bioavailable organic nutrients, failing to induce shifts in phosphorus cycling enzyme throughout the incubation period. (Line 261-272)

Comment: L250-257 – These results should be compared with other studies.

Response: OK. These results have been compared with other studies.

Manuscript text: Xu et al. (2024) reported similar results which showed that straw returning significantly improves the level of soil nutrient pool and enhances the availability of C matrix, thereby reducing the C limitation on microorganisms. The results of Zhang et al. (2025c) suggested that the addition rates of 1% and 2% of alkaline biochar alleviated the C limitation of soil microorganisms, while acid-modified biochar had no significant effect on vector length. (Line282-285)

Comment: L292 – The authors stated that “Applying straw and biochar at a 1:3 (straw:biochar) ratio effectively decreased the bioavailability of Cd and Zn (...)”. However, Fig. 1 shows that Cd content was not significantly different between treatments S1C3 and S0C4 at all 3 sampling times, and that Zn content was significantly lower in treatment S0C4 when compared with other treatments (including treatment S1C3) at 30 and 90 days.

I understand that this discrepancy arises because the authors used a different analysis in this subsection than in Fig. 1. However, I believe this should also be acknowledged and discussed.

Response: We apologize for the confusion we caused to our readers. We have revised this section to make it easier to understand and highlight the main purpose of this study.

Manuscript text: Applying straw and biochar at a 1:3 (straw:biochar) ratio balanced heavy metal remediation, carbon sequestration, nutrient availability, and microbial activity, thereby achieving the optimal comprehensive improvement of soil quality on Cd and Zn contaminated soil. (336-338)

Comment: Conclusions

L296 – The authors stated that “The combined application of straw and biochar effectively reduced (...) greenhouse gas emissions”. However, Fig. 2b shows that the lowest CO₂ emissions occurred in the control treatment (i.e., without biochar or straw) and in the S0C4 treatment (with biochar only).

Response: We highly appreciate the reviewer for pointing out this critical inconsistency. We agree with the reviewer’s observation. The previous generalized statement that “combined application reduced greenhouse gas emissions” was inaccurate and misleading. We have thoroughly revised the relevant statements in the manuscript to strictly conform to the actual results.

Manuscript text: The combined application of straw and biochar effectively remediated heavy metal pollution, increased soil organic carbon content and nutrient availability, and alleviated microbial nutrient stress. (341-342)