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.

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

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

Manuscript text: The chloroform fumigation method was used to determine microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP), calculated from the difference between the fumigated and non-fumigated samples (Hu et al., 2025a).

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.

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.

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, CO_2 emission and microbial biomass) has been clarified.

Manuscript text: Destructive sampling was conducted on days 30, 60 and 90 for each treatment.

The alkali absorption method (Geng et al., 2022) was used to measure the soil CO_2 emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90.

Microbial biomass was assessed using soil samples collected on day 90.

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: The available Cd content on days 30, 60 and 90 of CK treatment was 0.221 mg kg^{-1}, 0.220 mg kg^{-1}, and 0.230 mg kg^{-1}, respectively (Fig. 1a). On the 30th, 60th, and 90th days of incubation, the available Cd content of treatments containing both straw and biochar (S3C1, S2C2 and S1C3) and biochar alone (S0C4) was 0.197–0.143 mg kg^{-1}, 0.193–0.139 mg kg^{-1}, and 0.218–0.143 mg kg^{-1}, respectively, which were reduced by 10.60–35.34%, 12.30–37.04%, and 10.88–37.73%, compared to CK. On days 30, 60 and 90, the available Zn content of CK was 122.04 mg kg^{-1}, 121.62 mg kg^{-1}, and 120.66 mg kg^{-1}, respectively (Fig. 1b).

By day 90, the CO_2 emissions accumulation of CK and S0C4 was 1215.28 mg kg^{-1} and 1391.81 mg kg^{-1}, respectively.

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).

However, as the incubation experiment progressed, AHN content in straw-treated soil gradually decreased (Table S2). 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. This could be attributed to straw decomposition, which releases a large amount of available N (Zhao et al., 2026)

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}, respectively, which were the highest levels in each group.

For the treatment of 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.

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).

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.

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 CO_2 flux and accumulation was added.

Manuscript text: The alkali absorption method (Geng et al., 2022) was used to measure the soil CO_2 emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90.

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, CO_2 emission and microbial biomass) has been clarified.

Manuscript text: Destructive sampling was conducted on days 30, 60 and 90 for each treatment.

The alkali absorption method (Geng et al., 2022) was used to measure the soil CO_2 emission rate on days 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 90.

Microbial biomass was assessed using soil samples collected on day 90.

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). The extracted principal components could accurately represent the overall variation of soil heavy metal immobilization, nutrient supply, carbon sequestration and microbial functional properties of different treatments. 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.

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.

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; 200.0 g soil, no straw or biochar), (b) S4C0 (200.0 g soil and 4.0 g straw), (c) S3C1 (200.0 g soil, 3.0 g straw and 1.0 g biochar), (d) S2C2 (200.0 g soil, 2.0 g straw and 2.0 g biochar), (e) S1C3 (200.0 g soil, 1.0 g straw and 3.0 g biochar) and (f) S0C4 (200.0 g soil and 4.0 g biochar).

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)

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.

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; Hsiao et al., 2025). 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}, respectively, which were the highest levels in each group. S1C3 is the optimal ratio treatment to increase soil microbial biomass.

The reasons for S1C3 increasing soil microbial biomass are multifaceted. Biochar has unique advantages such as large specific surface area, developed pore structure, and strong adsorption performance (Chen et al., 2026). It can provide sufficient micro habitats for the reproduction, attachment, and growth of soil microbial communities, effectively protecting microorganisms from external environmental disturbances, improving soil microbial community abundance and stability, and indirectly promoting soil microbial nutrient accumulation (Jiang et al., 2025). Biochar improved soil available N, P, and K nutrients at the end of incubation (Fig. 5). However, biochar has significant limitations in C utilization. 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 (Chen et al., 2024b). It cannot provide an effective C source for microbial metabolism and proliferation processes, which significantly limits the growth of microorganisms under a single biochar treatment and restricts the increase in soil microbial biomass. Straw, as an organic material that is easily decomposed, can provide a sustainable C, N and P nutrients for microbial growth and reproduction after being applied to the soil (Ninkuu et al., 2025). It effectively relieves microbial nutrient limitations, especially C limitation, laying a good material foundation for the accumulation of soil microbial biomass. Heavy metal stress can inhibit soil microbial activity and reduce microbial biomass (Elrys et al., 2025). Therefore, the increase in soil microbial biomass was also partly attributed to the reduced biotoxicity of Cd and Zn Thus, combining straw and biochar, especially at the S1C3 ratio, was most effective in promoting microbial growth and increasing soil microbial biomass.

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 indicates 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 CO_2 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.

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, H., Guo, W., Li, G., Zheng, Y., and Zhou, J.: One-step waste biomass-derived K_2FeO_4-biochar for enhanced PCM thermal/photothermal performance. J. Environ. Chem. Eng., 14, 122075. https://doi.org/10.1016/j.jece.2026.122075, 2026.

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. 2025.

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.

Hsiao, C., Mushtaq, M., Sassenrath, G. F., Zeglin, L. H., Hettiarachchi, G. M., and Rice, C. W.: Long-term tillage and compost shape soil microbes under soil organic carbon equilibrium, Eur. J. Soil Sci., 76, e70125. https://doi.org/10.1111/ejss.70125, 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/frmbi.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.