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 (Line 29-30).

Manuscript text: Although zinc is an essential trace element for plants, excessive zinc can inhibit plant growth by reducing photosynthetic pigment content and affecting enzyme activity (Natasha et al., 2022).

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: Biochar alleviates the stress of heavy metals on soil microorganisms while increasing their nutrient requirements. However, its stable C structure limits its availability as a microbial carbon source, which may exacerbate nutrient limitations, thereby affecting microbial activity and overall soil ecology (Chen et al., 2022).

Compared with biochar, 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, the ability of single straw application to passivate heavy metals is limited.

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.

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.

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

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).

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).

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: 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%. An et al. (2025) showed that biochar and straw can partially replace chemical fertilizers and increase the content of soil available phosphorus and potassium, which may be because biochar and straw adsorb and release phosphorus and potassium. Ye et al. (2025) also indicated that biochar and straw can serve as phosphorus sources, gradually releasing phosphate through microbial activities, thereby increasing the available phosphorus content in the soil. Between S1C3 and S0C4, no significant differences were detected in soil AHN, Olsen-P and AK across all sampling periods.

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; 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: L233-234 – In my opinion, saying that " S4CO 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.

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-day incubation period (Table S3). 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. 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.

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

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-day incubation period (Table S3). 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. 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.

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% rates of alkaline biochar alleviated the C limitation of soil microorganisms, while acid-modified biochar had no significant effect on vector length.

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.

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 emmissions 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.