Response to Reviewer Jupei Shen

Thanks for your comments and suggestions. Please find our detailed answers below. Your comments are repeated in black, and our answers are in blue.

Comments from Reviewer

Rice straw incorporation is an important measure in the current sustainable development of agriculture, but its impact on greenhouse gas emissions remains controversial. The manuscript aimed to investigate the feasibility of straw incorporation to improve soil fertility without elevating global warming potential based on a microcosm experiment. This experiment has important practical value for optimizing fertilization strategies. Generally, it is an interesting topic, and the experimental design is rigorous. However, I have some concerns that should be addressed before the consideration for publication.

1. In the abstract, add the treatment information. Key data is also necessary in this part.

Response: We added the treatment information and key data to the abstract:

"*The utility of rice straw as an organic fertilizer has been widely recognized as a promising approach to enhancing soil fertility. However, straw return is currently in a dilemma, as it may also provoke greenhouse gas (GHG) emissions, leading to serious environmental consequences. It is urgent to reveal the feasibility of straw incorporation regarding soil fertility improvement without notable increases in GHG emissions. Here, a soil microcosm experiment was conducted using paddy soils collected from a long-term rice straw incorporation field experiment. The treatments of the field experiment, namely CK, ST1, ST2, and ST3 corresponding to the straw application rates of 0, 50%, 100%, and 150% of local rice straw yield, respectively, were continued in the microcosm experiment. The dynamics of GHG fluxes and concentrations in soils, and the variations in the abundances of soil microbial communities* *were systematically determined. The results indicated that ST1 treatment obviously improved soil fertility but did not induce significant elevation of global warming potential (GWP) with only 3% increase compared to CK. Although ST2 and ST3 treatments showed greater improvements in soil fertility, they significantly increased GWP by up to 151%. The minimal GWP increase under ST1 was mainly attributed to the significant reduction in N_2O emission and the slight rise in CH_4 emission compared to CK. The further investigations revealed that ST1 possessed the highest nosZII abundance and the lowest nirS/nosZII ratio, indicating its highest N_2O consumption ability. Meanwhile, its CH_4 production ability fluctuated around the soil CH_4 holding capacity (190 mmol·mol^{-1}), and most of the produced CH_4 was consumed by methanotrophs in soil. In conclusion, rice straw can be incorporated into paddy soils at a suitable application rate, which can effectively enhance soil fertility without inducing an additional warming effect*".

2. I would recommend to further point out the shortcomings of existing studies in terms of "synergistic effects of straw incorporation on both soil fertility and greenhouse gas emission, and the underlying mechanisms" in the Introduction section, which may help highlight the importance of the research.

Response: We added the shortcomings of existing studies in terms of "effects of straw incorporation on both soil fertility and GHG emissions, and the underlying mechanisms" in the Introduction (Line 55-57):

"*As a result, the balance and underlying mechanisms between soil fertility and GHG emissions are still poorly understood, leaving no clear consensus on how much straw should be incorporated appropriately*".

3. Provide a hypothesis for this study.

Response: We provided the hypothesis in the Introduction (Line 74-75): "*Here, we hypothesized that there would be a moderate straw incorporation rate, which could well balance improving soil fertility and minimizing the increase in GWP*".

4. L69, "from each treatment"

Response: Revised.

5. L85-87, the description of treatments is not clear. The straw treatments use the same amount of straw as did in the field experiment in the corresponding soils?

Response: The microcosm experiment used the same amount of straw as did in the field experiment in the corresponding soils. We converted the unit "kg straw·ha^{-1}" used in the field experiment into "g straw·kg^{-1} dry soil" for the microcosm experiment.

We revised the description (Line 117-119): "*The straw addition amounts in the incubation were 0, 2.00, 4.00, and 6.00 g straw·kg^{-1} dry soil for CK, ST1, ST2, and ST3 treatments, respectively, which were converted from the straw incorporation rates of the field experiment*".

6. L89, give more detail process for urea and straw application.

Response: The process was revised (Line 121-123): "*Urea was prepared as a stock solution (12.70 g N·L^{-1}), and 10 mL of this solution was added to each pot, equivalent to 100 mg N·kg^{-1} dry soil. After urea and straw were applied, the soil was thoroughly mixed immediately to ensure that the straw and N fertilizer can be uniformly distributed in the soil*".

7. Some detailed descriptions (such as gas collection devices) can be appropriately streamlined or moved to supplementary materials. Provide a schematic diagram of the cultivation device along with actual photos can give readers a better understanding.

Response: We streamlined the M&M, especially the text describing the gas collection devices. Please refer to the tracked changes. We also provided a photograph showing the pots during headspace gas sampling as Fig. S1b.

8. In the "DNA Extraction and qPCR" section, briefly explain the reasons for choosing these functional genes (such as nosZII type instead of nosZI type).

Response: We chose *nirS* and *nosZ*II was because they were reported to be more active than their paired genes of *nirK* and *nosZ*I in nitrite and nitrous oxide reduction processes, respectively, in paddy soils. In light of your Comment #9, we added the abundance data of *nirK* and *nosZ*I as Fig.S2.

9. It is recommended to provide the abundance data of *nirK* and *nosZ*I and also the correlation analysis between greenhouse gas fluxes and microbial gene abundance.

Response: We added the abundance data of *nirK* and *nosZ*I genes as Fig. S2, and the correlation analysis between GHG emissions and the corresponding microbial gene abundances as Table 3.

We briefly described the abundances of *nirK* and *nosZ*I genes:

Line 287-289: "*By comparison, the abundances of nirK declined after drainage and were less affected by straw incorporation than those of nirS (except ST3, Fig. S2)*".

Line 296-297: "*In contrast, the abundance differences of nosZI among treatments were far less pronounced than those of nosZII (Fig. S2)*".

10. In table 1, it would be better add the information for soils, which is derived from field samples.

Response: The soil information was added in the footnote of Table 1 (Line 203): "*These soils were collected from the plots of a long-term field experiment for the microcosm experiment*".

11. L226-227, "copies per g dry soil…"

Response: Revised.

12. Some statements in the discussion can be further simplified to avoid the repetition of the results, and recommend focusing more on the explanation of the mechanism and literature comparison.

Response: We revised the discussion following your suggestions.

13. If possible, supplement the discussion on the relationship between CO_2 emissions and soil carbon accumulation, such as: "Although the CO_2 emissions of ST1 treatment increased significantly, the soil organic carbon content also rose simultaneously, indicating that straw returning to the field promoted the turnover and stability of soil carbon."

Response: We supplemented the relationship between CO_2 emission and soil carbon accumulation in ST1 treatment (Line 323-325): "*Although the CO_2 emission of ST1 increased significantly compared to CK, its SOC content rose simultaneously, indicating this rate of straw returning was beneficial for the elevation of SOC*".

14. More relevant studies can be cited to enhance the persuasiveness in explaining N_2O emissions.

Response: The references below were cited to enhance the persuasiveness in explaining N_2O emission:

Cai, Y., Zhang, H., Hu, X., Yang, Y., Hazard, C., Nicol, G. W., He, J., Shen, J., He, Z., Zhang, L., Zhang, J., Liu, H., Zhang, S., and Chen, Z.: Millimeter-scale niche differentiation of N-cycling microorganisms across the soil-water interface has implications for N_2O emissions from wetlands, ISME J., 19, wraf062, https://doi.org/10.1093/ismejo/wraf062, 2025.

Chiriac, O. P., Pittarello, M., Moretti, B., and Zavattaro, L.: Factors influencing nitrogen derived from soil organic matter mineralisation: Results from a long-term experiment, Agric. Ecosyst. Environ., 381, 109444, https://doi.org/10.1016/j.agee.2024.109444, 2025.

Cucu, M. A., Said-Pullicino, D., Maurino, V., Bonifacio, E., Romani, M., and Celi, L.: Influence of redox conditions and rice straw incorporation on nitrogen availability in fertilized paddy soils, Biol. Fertil. Soils, 50, 755-764, https://doi.org/10.1007/s00374-013-0893-4, 2014.

He, G., Wang, W., Chen, G., Xie, Y., Parks, J. M., Davin, M. E., Hettich, R. L., Konstantinidis, K. T., and Löffler, F. E.: A novel bacterial protein family that catalyses nitrous oxide reduction, Nature, 646, 152-160, https://doi.org/10.1038/s41586-025-09401-4, 2025.

Wang, L., Xu, H., Liu, C., Yang, M., Zhong, J., Wang, W., Li, Z., and Li, K.: Stronger link of nosZI than nosZII to the higher total N_2O consumption in anoxic paddy surface soils, Geoderma, 425, 116035, https://doi.org/10.1016/j.geoderma.2022.116035, 2022.

Zhou, W., Jones, D. L., Hu, R., Clark, I. M., and Chadwick, D. R.: Crop residue carbon-to-nitrogen ratio regulates denitrifier N_2O production post flooding, Biol. Fertil. Soils, 56, 825-838, https://doi.org/10.1007/s00374-020-01462-z, 2020.

15. In the discussion part, add some comments for the abundance of detected genes compared to literature.

Response: We compared gene abundances with literature (Line 360-363): "*Moreover, the microbial gene abundances in this study were at the higher end of the ranges reported in the literature (Ma et al., 2012; Zhou et al., 2020b; Wang et al., 2022; Yi et al., 2024; Cai et al., 2025; Zheng et al., 2026). This could be attributed to the differences in soil properties among studies and to straw incorporation that provided substrates for microbial growth*".

16. It is also recommended to add the limitations of this research.

Response: We revised the limitations of our research (Line 369-370): "*Therefore, the field tests with different soil types are necessary*".

17. The manuscript would benefit from further linguistic refinement.

Response: We thoroughly checked and polished our grammar, syntax, coherence, and cohesion with the help of native speakers. Please refer to the tracked changes in the revised MS.