Response to Reviewer Jun Shan

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

In this study, the authors claimed that rice straw could be incorporated into paddy soils by optimizing the straw rate and explained why the optimal rate minimized global warming potential mainly from the functional microbial perspective. The topic is interesting and well within the scope of SOIL journal. The MS is generally well-organized and well-written, and the methodology is sound. However, I have several comments to improve the quality of the MS. I recommend **major revision before acceptance**.

General comments

1. The first concern is about the title. The title is overstated. Investigating feasibility can include several aspects such as agronomic measures, soil types, climate regions, and land use, while the authors only focus on the rice straw rate and one paddy soil. I recommend reconsidering the title.

Response: The revised title is: *"Mechanisms of enhancing soil fertility without obviously elevating global warming potential under optimal rice straw incorporation rate in a paddy soil"*.

2. The authors used paddy soils from a long-term field to investigate GHG emission and microbial abundances. I acknowledge that the incubation approach has distinct advantages especially in elucidating the mechanism. The authors should claim more about why they chose to further conduct the microcosm experiment by following the field setting instead of directly sampling from the field.

Response: The reason for us to use microcosm experiment was aiming to precisely measure the dynamics of soil GHG concentration and emission and investigate the mechanisms under controlled environment. Because the conditions in the field (i.e., temperature, rainfall, and field managements) are variable, which can seriously affect the measurements, we could hardly achieve our study aims. So, the soils from the plots of a long-term field experiment with five-year rice straw incorporation were used for the microcosm experiment.

The reason for conducting the microcosm experiment was added (Line 75-77):

"Considering that field experiments can hardly avoid the influences of climate and other factors, and the advantages of microcosm experiment including controlled environment and high operability in frequent gas and soil sampling, …"

3. The authors should explain more about why they only focused on *nirS* and *nosZ*II while excluding their paired genes *nirK* and *nosZ*I. I suggest adding one or two sentences in the Method section to clarify this.

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. As another referee suggested, we added the abundance data of *nirK* and *nosZ*I as Fig.S2.

4. The authors revealed that the soil has a holding capacity for CH_4 while not mentioning this for N_2O. From the concentration data, it seems soil did not hold N_2O at least not as long as CH_4. Why? It would be interesting if authors could explain a bit about this since the soil holds CH_4 while not holding N_2O may have implications for GHG mitigation strategy.

Response: Based on our observations, the soil showed a CH_4 holding capacity under anaerobic conditions. It would be related to weak anaerobic CH_4 oxidation activity and soil properties. The mechanisms for this phenomenon require further investigations. However, we did not detect such a result for N_2O. The possible reason would be linked to strong complete denitrification activities under anaerobic conditions, where N_2O reducers could efficiently reduce the produced N_2O. Therefore, N_2O could hardly accumulate and the holding capacity of N_2O was not detected.

5. Besides CH_4 and N_2O, CO_2 is also an important GHG as it contributed up to 45% to the GWP (Table 2). I recommend elaborating more on the mechanisms related to CO_2 in the Discussion part.

Response: The discussion was revised (Line 320-325) as follows:

"*It was detected that the CO_2 fluxes were positively related to the straw incorporation rate, and the higher input induced the higher CO_2 emission. Among straw–amended treatments, ST1 generated the lowest cumulative CO_2 flux, which was about 1.7 times of CK. This would be linked to the small amount of organic substrate for the soil microbial communities and the limited growth of microbial population (Miller et al., 2008; Li et al., 2022). 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*".

6. Please improve your English and check the grammar throughout the paper.

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.

Specific comments

Line 55-56. "This study aimed to…, and the related mechanisms". I suggest rephrasing this sentence.

Response: This sentence was refined (Line 80-81): "*This study aimed to reveal the mechanisms of boosting soil fertility without elevating GWP under an optimal rice straw incorporation rate*".

Line 66. Please add the description about water management of the field experiment since water regime could have a legacy impact on GHG emissions. This information would be valuable for potential readers interested in relevant topics.

Response: We added the information about the water management of the field experiment (Line 91-94): "*All treatments were subjected to the same water management. During the rice season, all plots were continuously flooded except for two drying events. The first drying event occurred at the end of the tillering stage and lasted for one to two weeks. The second drying event happened one week before harvest. In the fallow season, all plots remained unirrigated*".

Line 77-83. You sampled the headspace gas in the chamber and then the soil gas in the silicone tube (see Line 96-100). For consistency, I suggest adjusting the order here: For gas flux sampling, … For soil gas sampling, …

Response: Reordered.

Line 84-85. "and each pot contained 1.27 kg (dry weight) of soil to reach about 10 cm in depth". I suggest revising this sentence.

Response: We revised this sentence (Line 116-117): "*Each pot was filled with wet soil to 10 cm in height containing 1.27 kg of dry soil*".

Line 99. What does "at the same time" mean? Did you sample headspace gas and soil gas simultaneously?

Response: This wording is inaccurate. We both sampled headspace gas and soil gas at each sampling time. During each sampling, we followed the order of "headspace gas"-"soil gas". We modified the text (Line 132-133): "*Immediately after collecting all headspace gas samples, 1 mL of soil gas was taken from each buried silicone tube, with an equal volume of helium injected back*".

Line 193-195. The sentence is too long. Please revise it.

Response: The sentence was modified (Line 231-233): "*The dynamics of N_2O emission exhibited two distinct peaks, with one occurring shortly after fertilization and the other during the late drying period (Fig.1c). However, the variation patterns of the two peaks differed markedly both among treatments and between periods*".

Line 249. "similar" should be "similarly".

Response: Revised.

Line 266. It is inappropriate to use “fail to” here. We don’t expect an increase.

Response: The sentence was revised (Line 315-316): “*More importantly, ST1 treatment did not cause a significant increase in GWP compared to CK*”.

Line 291. I suggest deleting “with less than 5 g·m^{-2}”. It is meaningless to point out the number here. The GHG cumulative emissions from microcosms are incomparable among studies.

Response: The sentence was revised (Line 348-349): “*Interestingly, the cumulative CH_4 emission from ST1 treatment remained low, whereas ST2 and ST3 sharply elevated the emissions to about 5 and 9 times that of ST1, respectively*”.

Line 293. Change “such a low” to “a much lower”, as the CH_4 cumulative emission should be only compared to the three other treatments in THIS study.

Response: The sentence was deleted following the suggestion from Referee #1.

Line 298-300. Grammatical errors. Please revise the whole sentence.

Response: The sentence was revised (Line 354-355): “*Soil CH_4 consumption would be strongly linked to soil CH_4 holding capacity (Ariani et al., 2022), as the retained CH_4 can be oxidized by methanotrophs*”.

Line 312-314. For now, the outlook could be more comprehensive. Consider revising this if possible.

Response: Here is the revised outlook (Line 369-370): “… *Therefore, the field tests with different soil types are necessary*”.

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 the 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.

Response to Anonymous Referee #1

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

This study used a soil microcosm experiment to investigate the effects of straw incorporation on the relationship between soil fertility and greenhouse gas (GHG) fluxes, as well as the underlying microbial mechanisms. These are relevant and timely topics in the context of straw return and GHG mitigation. However, several issues need to be addressed before the manuscript can be considered for publication.

Introduction

1, The introduction mainly lists the effects of straw incorporation on CO2, CH4, and N2O emissions, but lacks a sufficient synthesis of the underlying regulatory mechanisms, particularly the microbially mediated processes involved in greenhouse gas production and consumption. As a result, the knowledge gap identified in the current version remains rather broad.

Response: We added a paragraph introducing the underlying microbial regulatory mechanisms governing GHG production and consumption under straw incorporation (Line 59-73):

"*Soil GHG emissions are predominantly regulated by the microbially mediated production and consumption processes. CH_4 production and oxidation are catalyzed by methyl-coenzyme M reductase of methanogens and particulate methane monooxygenase of methanotrophs, with their active subunits encoded by mcrA and pmoA genes, respectively (Gao et al., 2020; Liu et al., 2025). The potential for N_2O production in paddy soils is closely associated with nitrite reduction, with nitrite reductases encoded by nirK and nirS genes (Chen et al., 2010; Chen et al., 2024; Kong et al., 2024). N_2O reduction is typically mediated by two distinct clades of N_2O reductase, encoded by nosZI and nosZII genes,* *respectively (Jones et al., 2013; Hallin et al., 2018; Liu et al., 2023a; Yang et al., 2024). While straw incorporation generally enhanced CH_4 production, its effects on CH_4 oxidation and N_2O production and reduction remain controversial. Some studies reported negligible effects of straw application on pmoA abundance (Wang et al., 2018), but others found increases in pmoA abundance (Jiang et al., 2019; Zhou et al., 2020a). For instance, long-term straw amendment increased pmoA abundance by 59% (Yang et al., 2022). Likewise, straw incorporation led to inconsistent responses from N_2O-related genes (Wang et al., 2018; Wang et al., 2021). Specifically, the abundances of nirK, nirS, nosZI, nosZII was reported to increase, decrease, or remain unchanged following straw application (Wang et al., 2018). Taken together, the microbial mechanisms underlying GHG responses to straw incorporation remain insufficiently understood, particularly how key functional microbial communities respond to different straw application rates and thereby regulate GHG emissions*".

2, Although the study objective is stated at the end of the introduction, it has not been further distilled into clear scientific questions, and corresponding hypotheses are also lacking. The authors are therefore encouraged to explicitly state the scientific questions addressed in this study and to propose relevant hypotheses at the end of the introduction.

Response: We refined the study objective (Line 80-81): "*This study aimed to reveal the mechanisms of boosting soil fertility without elevating GWP under an optimal rice straw incorporation rate*".

We also added the hypothesis (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*".

3, This study further conducted an indoor incubation experiment based on a 5-year field trial; however, the necessity of this design and its advantages over field observations have not been sufficiently explained in the introduction. The authors are advised to clarify the logical connection between the long-term field background and the subsequent indoor mechanistic investigation.

Response: The reason for us to use microcosm experiment was aiming to precisely measure the dynamics of soil GHG concentration and emission and investigate the mechanisms under controlled environment. Because the conditions in the field (i.e., temperature, rainfall, and field managements) are variable, which can seriously affect the measurements, we could hardly achieve our study aims. So, the soils from the plots of a long-term field experiment with five-year rice straw incorporation were used for the microcosm experiment.

The reason for conducting the microcosm experiment was added (Line 75-77):

"Considering that field experiments can hardly avoid the influences of climate and other factors, and the advantages of microcosm experiment including controlled environment and high operability in frequent gas and soil sampling, …"

Materials and Methods

1, It is suggested that Section 2.1 be divided into two subsections: 2.1 Site description and experimental design and 2.2 Soil and straw sampling and preparation.

Response: Revised.

2, Line 64: Please clarify the physical condition of the straw (e.g., whether it was chopped or crushed, and its length range) and the depth at which it was incorporated. In addition, please explain why the rice straw was sieved through a 1 mm mesh during the incubation experiment (Line 73), and why the straw was fully mixed with the 0-15 cm soil layer (Line 89) instead of following the actual field situation.

Response: We added the physical condition of the straw and the depth at which it was incorporated (Line 88-90): "*Rice straw cut into 10 cm segments was evenly spread on the surface of the corresponding plots and turned over into the plow layer (0–20 cm) in each early May*".

We explained why we sieved the straw (Line 104-105): "*The sieved straw can be evenly distributed and contacted with soil particles, generating similar* *decomposition speeds among the pots of each treatment"*.

The reason why the straw was fully mixed with soil instead of following the actual field situation is that field experiments rely on relatively large areas to reduce errors caused by management. However, microcosm experiments only use a limited amount of soil. To minimize errors, the straw and other fertilizers have to be mixed thoroughly with soil. We explained this (Line 122-123) as follows: "*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*".

3, Line 71: Please specify the temperature conditions used during the pre-incubation period.

Response: We specified the temperature conditions (Line 101-102): "*…, and the resulting soil slurries were pre-incubated under flooding at room temperature for 30 days*".

4, Line 77: It would be helpful to add a photo of the experimental setup in the Supplementary Materials. Together with the schematic diagram, this would make the experimental procedure clearer.

Response: We provided a photograph showing the pots during headspace gas sampling as Fig. S1b.

5, Line 84: Why was 1.27 kg of soil used? Please clarify whether this amount was determined according to the actual field bulk density.

Response: Based on our pre-experiment, filling the pot with slurry to 10 cm in height corresponded to 1.27 kg of dry soil. The density in the pot may not be the same as the field bulk density. We clarified this (Line 116-117): "*Each pot was filled with wet soil to 10 cm in height containing 1.27 kg of dry soil*".

6, Line 96: Was the sampling conducted at a fixed time each day, for example, 9:00–10:00 a.m.? Please specify the sampling time and briefly explain the reason for this setting.

Response: We collected gas samples between 9:00 and 11:00 AM during each sampling event. The gas samples collected within this time window are generally considered the most representative of the daily mean emission level.

We specified the sampling time (Line 129): "*Gas samples for GHG fluxes and soil profile concentrations were taken between 9:00 and 11:00 AM every 1–3 days*".

7, Line 99: Please clarify the purpose of taking 1 mL of gas from the silicone tube buried at the bottom, including what parameter it was used to measure or what role it played in the experimental design.

Response: Taking 1 mL of gas from the silicone tube buried at 5 cm in depth (in the middle) was to reflect soil concentrations. The gas was used to measure CO_2, CH_4, and N_2O concentrations. We clarified this (Line 133-134): "*The gas from the silicone tube was used to determine the soil CO_2, CH_4, and N_2O concentrations*".

Results

1, According to the study objectives, soil fertility is one of the core concerns. However, the related results are currently mainly included under 3.1 Soil properties, where they are not sufficiently highlighted. The authors are suggested to strengthen the presentation of soil fertility-related results and consider reflecting this aspect more clearly in the subsection title.

Response: We revised the subsection title as "*3.1 Influence of straw incorporation on soil fertility*" (Line 192).

We strengthened the presentation of soil fertility-related results (Line 193-201): "*Soil physicochemical characteristics were clearly affected by continuous straw incorporation at various rates for five years (Table 1). The results indicated that soil BD was negatively and significantly correlated with the rice straw incorporation rate ($p < 0.05$), suggesting the physical character was improved. Concerning the detected soil chemical properties, except for TP, TK, and Olsen P, which were not clearly influenced by straw incorporation, the rest properties were positively related to the straw application rate. Among them, the variations* *of CEC ($p < 0.05$), AvK ($p < 0.001$), and AvZn ($p < 0.05$) were significantly affected. When compared with CK, even the least amount of straw input (ST1) induced the decrease of BD by 2.76% and the increases of CEC, TC, TN, AvN, Olsen P, and AvK by 3.13%, 4.19%, 2.58%, 6.83%, 15.09%, and 38.52%, respectively. ST1 treatment also resulted in obvious increases in soil micronutrient concentrations in comparison with CK, with the contents of AvMn, AvCu, and AvZn elevated by 18.48%, 4.57%, and 8.09%, respectively*".

2, No correlation analysis was conducted, yet terms such as "positively related to" and "negatively related to" are used in the Results section. Such wording is not rigorous without statistical support. The authors are advised to either perform correlation analysis or revise these statements to more descriptive expressions.

Response: We added the correlation analysis between soil properties and straw rate in Table 1. We also added the correlation analysis between straw rate, GHG emissions, and microbial abundances as Table 3.

3, The incubation experiment included both flooding and drainage periods, and Fig. 1 shows that both CO_2 and CH_4 emissions peaked in each stage. However, the Results section does not describe these two periods separately. Since the experimental design clearly distinguished them, the authors are encouraged to further compare the emission patterns between the flooding and drainage periods. In addition, it is suggested that cumulative emissions in Table 2 be calculated separately for the two stages to better reflect their differences.

Response: We agree that comparing emission patterns between flooding and drainage periods is informative. However, the overall cumulative emissions in Table 2 integrate the contrasting emission dynamics during both stages and are thus considered the most comprehensive indicator for evaluating the warming effects of straw incorporation. As suggested, we presented the stage-specific emission data as Table S2. This provides a more detailed emission picture while retaining the overall cumulative emissions as the primary metric.

Discussion

1, The expression “We hypothesized that …” in Line 264 is not appropriate in the Discussion section. Research hypotheses should normally be presented in the Introduction rather than introduced for the first time in the Discussion. The authors are advised to move the hypothesis to the end of the Introduction and then discuss whether it is supported by the results.

Response: The hypothesis was moved into the Introduction (Line 74-75). The Discussion was originally structured around the hypothesis, and we further clarified this connection in the revised MS.

2, The discussion of soil fertility is still insufficient. At present, the effect of ST1 on soil fertility is only briefly mentioned in one sentence (Line 266), which does not fully respond to the study objectives. The authors are encouraged to specify which soil fertility-related indicators were improved by ST1 and to further analyze the possible reasons based on the results.

Response: Agree. The discussion was revised (Line 304-315) as follows:

“*Soil fertility is the capability of soil to continuously supply water, nutrients, air, and suitable thermal conditions for plant growth, which can be reflected by soil physical, chemical, and microbial characteristics (Theresa et al., 2026). We found that soil BD was negatively related to the straw incorporation rate, and soil CEC and soil available nutrient concentrations were positively related to the rate. The results strongly suggested that five-year rice straw incorporation improved soil fertility by increasing soil aeration, soil cation holding capacity, and nutrient supply ability (Xing et al., 2025). In this study, the least amount of straw incorporation (ST1) generated the lowest GWP among straw–amended treatments. If this treatment can also lead to the elevation of soil fertility, it would support our hypothesis. The results showed that although the soil physicochemical properties of ST1 were not significantly different from those of CK, the characteristics were systematically improved. For instance, BD decreased by 2.76%, and TC and AvK increased by 4.19% and 38.52%, respectively. It is obvious that after five years of rice straw incorporation under ST1, soil porosity, soil organic matter, and nutrient supply ability all increased, indicating its soil fertility was improved*”.

3, The reason why ST1 did not significantly increase GWP is not clearly discussed. According to Table 2, although ST1 significantly increased the cumulative emissions of CO2 and CH4, it also significantly reduced N2O emissions, which likely explains why GWP did not increase markedly. This point should be stated more explicitly in the Discussion, together with the relative contributions of different gases to GWP. At present, the manuscript only discusses the reason for the increase in N2O under CK, while the overall effect of ST1 is not sufficiently addressed.

Response: Agree. The discussion was revised (Line 315-319) as follows:

"*More importantly, ST1 treatment did not cause a significant increase in GWP compared to CK. It was observed that the cumulative CO_2 and CH_4 emissions of ST1 were significantly higher than those of CK, but the cumulative N_2O emission was significantly lower. Since the warming potentials over a 100-year horizon of CO_2, CH_4, and N_2O are 1, 27.9, and 273, respectively (Forster et al., 2021), the major contributor to the low GWP under ST1 would be the significantly lower N_2O emission*".

4, If the authors intend to emphasize the regulatory role of microbial changes in GHG or GWP, corresponding correlation analyses are recommended.

Response: Agree. The correlation analysis between GHG emissions and microbial gene abundances was added as Table 3.

5, Line 293: There is a grammatical problem in this sentence. "Such as" should not be followed by a complete sentence, and the sentence structure should be revised.

Response: The sentence was revised (Line 348-349): "*Interestingly, the cumulative CH_4 emission from ST1 treatment remained low, whereas ST2 and ST3 sharply elevated the emissions to about 5 and 9 times that of ST1, respectively*".

6, Line 294: The phrase "The question is why …" is rather colloquial and adds little information. It is suggested to delete this expression.

Response: It was deleted.

Response to Anonymous Referee #2

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

Introduction

1, L38–45: The reported effects of rice straw incorporation on CH_4, CO_2, and N_2O emissions vary substantially across studies. As currently written, it is unclear whether this variability is primarily driven by differences in the amount of straw incorporated or by other environmental and management factors (e.g., soil properties, water management, climate, or fertilization practices). While the introduction indicates that reducing straw input may be a promising strategy for mitigating GHG emissions, it would be helpful to briefly discuss the mechanisms through which straw incorporation influences GHG fluxes. Providing this background would strengthen the rationale for investigating straw application rates and help justify the objectives of the study.

Response: We added the potential importance of straw rate as a contributor to variable GHG emission responses (Line 49-51): "*The variability in GHG emissions could be affected by several factors, such as soil properties, agricultural management, and climate conditions, with the amount of straw incorporated potentially playing a vital role*".

Additionally, we added a paragraph introducing the underlying microbial regulatory mechanisms governing GHG production and consumption under straw incorporation (Line 59-73):

"*Soil GHG emissions are predominantly regulated by the microbially mediated production and consumption processes. CH_4 production and oxidation are catalyzed by methyl-coenzyme M reductase of methanogens and particulate methane monooxygenase of methanotrophs, with their active subunits encoded by mcrA and pmoA genes, respectively (Gao et al., 2020; Liu et al., 2025). The* *potential for N_2O production in paddy soils is closely associated with nitrite reduction, with nitrite reductases encoded by nirK and nirS genes (Chen et al., 2010; Chen et al., 2024; Kong et al., 2024). N_2O reduction is typically mediated by two distinct clades of N_2O reductase, encoded by nosZI and nosZII genes, respectively (Jones et al., 2013; Hallin et al., 2018; Liu et al., 2023a; Yang et al., 2024). While straw incorporation generally enhanced CH_4 production, its effects on CH_4 oxidation and N_2O production and reduction remain controversial. Some studies reported negligible effects of straw application on pmoA abundance (Wang et al., 2018), but others found increases in pmoA abundance (Jiang et al., 2019; Zhou et al., 2020a). For instance, long-term straw amendment increased pmoA abundance by 59% (Yang et al., 2022). Likewise, straw incorporation led to inconsistent responses from N_2O-related genes (Wang et al., 2018; Wang et al., 2021). Specifically, the abundances of nirK, nirS, nosZI, nosZII was reported to increase, decrease, or remain unchanged following straw application (Wang et al., 2018). Taken together, the microbial mechanisms underlying GHG responses to straw incorporation remain insufficiently understood, particularly how key functional microbial communities respond to different straw application rates and thereby regulate GHG emissions*".

2, Please add hypotheses to the end of the introduction section.

Response: Agree. The hypothesis was added (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*".

Materials and Methods

L64-65: If available, it would be useful to include the depth of rice straw incorporation in the field plots from which soils were collected.

Response: We added the depth of rice straw incorporation in the field plots (Line 88-90): "*Rice straw cut into 10 cm segments was evenly spread on the surface of the corresponding plots and turned over into the plow layer (0–20 cm) in each early May*".

L65: The word "Besides" is not needed in this sentence.

Response: The word "Besides" was deleted.

L70-71: It is unclear whether plant residues and gravel were removed manually or by sieving. If sieving was used, please specify the sieve size.

Response: The revision (Line 99-102) is as follows: "*The fresh soils were directly flooded and homogenized into slurries to avoid the impact of air–drying on microbial communities. Plant residues and gravel were removed by sieving the slurries through a 2 mm mesh, and the resulting soil slurries were pre-incubated under flooding at room temperature for 30 days*".

L84: Please provide justification for the use 1.27 kg (dry weight) per pot – it seems it may be to reach 10 cm in depth, but the way it is written it is not entirely clear.

Response: Based on our pre-experiment, filling the pot with slurry to 10 cm in height corresponded to 1.27 kg of dry soil. We clarified this (Line 116-117): "*Each pot was filled with wet soil to 10 cm in height containing 1.27 kg of dry soil*".

Results

L162-163: The relationships between TN, TC, available nutrients and CEC and straw incorporation rate are described as being 'positive', while BD was described as 'negatively related' to the rate. This terminology implies correlation – if a correlation analysis was conducted, it should be included in the Materials and Methods section.

Response: We described the analysis method in the Materials and Methods section (Line 189-190): "*Spearman correlation analysis was conducted using corrplot package in R v4.5.3 (α = 0.05, 0.01, or 0.001)*".

Discussion

L263: The hypothesis should be moved to the *Introduction* section. It should also be stated more explicitly. In addition, the hypothesis would benefit from greater specificity. For example, were any of the treatments expected to improve soil fertility while minimizing increases in global warming potential (GWP)? Providing specific predictions would strengthen the rationale of the study.

Response: We moved the hypothesis to the Introduction. The revised hypothesis (Line 74-75) is as follows: "*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*".