# 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 rice straw incorporation rates were negatively and significantly correlated with soil BD ($p < 0.05$), 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 the 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 analyses between soil properties and straw rate in Table 1. We also added the correlation analyses 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 CO2 and CH4 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 increased about 4.19% and AvK elevated by approximately 38.52%. 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.