Response to Anonymous Referee #1

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

Comments from Reviewer

The author has responded to and revised all the issues mentioned in the previous review point by point. In the Introduction, the mechanisms by which straw return regulates greenhouse gas emissions were elaborated, the scientific questions and research hypotheses of this study were clarified, and the necessity of the laboratory incubation experiment was illustrated. In the Materials and Methods section, experimental details were supplemented and revised. In the Results section, the analysis and description of the effects of straw return on soil fertility indicators were strengthened, correlation analysis results were newly added, and stage-specific data of greenhouse gases were presented in phases. In the Discussion section, the mechanistic analysis regarding how straw return affects soil fertility was supplemented and improved. All revisions are thorough and appropriate, and the manuscript meets the publication requirements. I recommend acceptance.

Response: We sincerely appreciate your time and effort on our MS.

Response to Referee #3 Abel Veloso

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

Comments from Reviewer

In my opinion, the paper entitled "Mechanisms of enhancing soil fertility without obviously elevating global warming potential under an optimal rice straw incorporation rate in a paddy soil" is well written and approaches a subject that is both scientifically relevant and well within the journal's scope.

My main concerns are related with the interpretation of results under the light of statistical analysis. In my opinion, the authors should be more strict while inferring the possible effects of treatments on the response variables. My specific comments are detailed below.

Introduction

L77 - I believe that the authors meant "a soil microcosm experiment" (or something equivalent).

Response: It was revised as "*a soil microcosm experiment*" (Line 77).

Materials and methods

L178 – What were the main reasons behind this selection?

Response: The main reasons for this selection was demonstrated in the Introduction (Line 59-65).

Results

Error bars should be included in the figures. Additionally, the authors stated repeatedly in each figure legend that “Data are given in average ± standard error”, which obviously does not apply to a figure.

Response: Error bars were included in all figures, but some of them were too small to be visible. We recolored all error bars black to improve visibility. Please refer to the revised figures.

Subsection 3.1 Influence of straw incorporation on soil fertility (L192-209)

This subsection should be reformulated considering the following aspects:

The influence of treatments on response variables should rely mainly on ANOVA + Tukey HSD (or equivalent tests) and not on Spearman correlations. The latter should be used to evaluate possible relationships/correlations among response variables.

No influence of treatments on response variables should be inferred in the absence of significant statistical differences. For example, the authors stated that (L195-197) “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.” However, the ANOVA results only indicate significant differences among treatments in BD, CEC,. AvK and AvZn.

Related to this (L198-199) the authors stated that “When compared with CK, even the least amount of straw input (ST1) induced the decrease of BD by 2.76%.” However, the letter in Table 1 indicate that no significant differences can be found between the effect of those 2 treatments on BD”.

Response: We used ANOVA + Tukey HSD/GH tests to interpret the influence of treatments on soil properties, and Spearman correlation to evaluate the associations between straw incorporation rate and soil properties.

As soils were sampled during the fallow season after only five years of the ongoing field experiment, the relatively high environmental variability under field conditions may explain why most variables did not reach statistical significance among treatments. Considering the cumulative effects of straw incorporation on soil properties, we expect these differences to become statistically significant with longer experimental duration.

Notably, AvK has exhibited significant differences among treatments, directly reflecting the effect of straw incorporation. For BD, CEC, AvN, AvMn, AvCu, AvZn, TC, and TN, although no significant differences were detected, all indicators showed a tendency toward improvement relative to CK; and most tended to improve with increasing straw incorporation rates. Taken together, these results demonstrated the beneficial effects of straw incorporation on soil properties.

To clarify, we revised as follows:

Line 194-197: "*The results indicated that soil BD was negatively and significantly correlated with the rice straw incorporation rate ($p < 0.05$), and soil pH, CEC ($p < 0.05$), AvN, AvK ($p < 0.001$), AvMn, AvCu, AvZn ($p < 0.05$), TC, and TN were positively related to the straw application rate. Although most of the characteristics were not statistically significant among treatments under field conditions, …*".

Line 200-201: "*These data suggested an improving trend in soil fertility resulting from rice straw incorporation*".

Figure and table legends should include more information.

Response: We added necessary information to the legends of figures and tables. Please refer to the revisions.

L231-232 – Fertilisation events should be included on the plot.

Response: Urea and straw were added before the start of the microcosm experiment (Line 121-123).

We revised the sentence (Line 233-234): "*The dynamics of N_2O emission exhibited two distinct types of peaks, with one type peaking as early as day 11 after fertilization and the other during the late drying period (Fig.1c)*".

L233-234 – The authors referred that "During the first peak, CK emitted most N2O (..)". However, the CO2 flux emmited by CK during that period is much higher than N2O flux. I believe that what the authors meant was that during the 1st peak, CK was the treatment emmiting the highest N2O flux.

Response: We did not intend to compare emission differences among GHGs. Here, we focused on N_2O emissions among treatments and meant that during the 1st peak, CK was the treatment emitting the highest N_2O flux.

Subsection 3.3 – I would suggest that the authors test the effects of treatments on cummulative emmissions and GWP.

Response: The effects of treatments on cumulative emissions and GWP were presented in Table 2 within Subsection 3.2, as both variables are calculated directly from the flux data and are more appropriately analyzed alongside the flux patterns. In Subsection 3.3, we focused on GHG concentrations in soils to interpret the flux results.

To clarify, we revised the title of Subsection 3.2 (Line 210) as "*Influence of straw incorporation on GHG emissions and global warming potential*".

L261 – Similarly to what I referred above, the authors should only consider significant differences among treatments to order them. Apart from this, the authors statement is not applicable to days 28, 42 and 70.

Response: We revised and expanded Subsection 3.4.1 (Line 264-273): "*Across the six sampling days, the average copy numbers of 16S rRNA were 3.92×10^{12}, 4.50×10^{12}, 4.76×10^{12}, and 5.71×10^{12} per g dry soil for CK, ST1, ST2, and ST3, respectively (Fig. 3). The corresponding values for 18S rRNA were 2.04×10^{9}, 2.78×10^{9}, 3.03×10^{9}, and 3.63×10^{9} per g dry soil, respectively. The bacterial and fungal population sizes exhibited similar temporal dynamics during the incubation. They were obviously affected by straw incorporation on day 14 under flooding, when ST2 and ST3 induced significantly higher abundances of bacteria and fungi than CK, but no significant differences were detected between ST1 and CK. Afterwards, the populations of bacteria and fungi fluctuated among treatments until day 56 during the early drained phase, on which the influence of straw incorporation on the microbial abundances appeared again and ST3 showed significantly higher population sizes of bacteria and fungi than CK. During the late drained period on days 70 and 84, this influence continued, where ST3 possessed significantly larger* *bacterial populations than CK, and the three straw–amended treatments resulted in significantly higher fungal abundances than CK*".

L262 – Please change numbers to scientific notation (e.g. 3.92×10^{12})

Response: Revised.

Subsection 3.4.1 should be expanded.

Response: We expanded Subsection 3.4.1 (Line 266-273): "*The bacterial and fungal population sizes exhibited similar temporal dynamics during the incubation. They were obviously affected by straw incorporation on day 14 under flooding, when ST2 and ST3 induced significantly higher abundances of bacteria and fungi than CK, but no significant differences were detected between ST1 and CK. Afterwards, the populations of bacteria and fungi fluctuated among treatments until day 56 during the early drained phase, on which the influence of straw incorporation on the microbial abundances appeared again and ST3 showed significantly higher population sizes of bacteria and fungi than CK. During the late drained period on days 70 and 84, this influence continued, where ST3 possessed significantly larger bacterial populations than CK, and the three straw–amended treatments resulted in significantly higher fungal abundances than CK*".

Figure 4 – Abbreviation meanings should be included in the legend.

Response: *mcrA* and *pmoA* are genes encoding the active subunits of methyl-coenzyme M reductase of methanogens and particulate methane monooxygenase of methanotrophs, respectively. The abundances of the two genes were used to indicate CH_4 production and oxidation potential, respectively. The *mcrA*/*pmoA* ratio was used to reflect the relative abundance of the two microbial groups. The introduction to the two genes was in Line 59-62.

L272-273; L274-276; L277-278 – In what day(s)?

Response: These results refer to the average data during CH_4 fluxing period between day 14 and day 56. We revised the relevant sentences as follows:

Line 283: "*…, the least average increase occurred in ST1 with 32%, …*".

Line 285-287: "*…, the treatment with the lowest amount of straw (ST1) induced a substantial increase in methanotrophs compared to CK by approximately 81% on average between day 14 and day 56 (Fig.4b)*".

Line 289: "*…, the average ratio of ST1 between day 14 and day 56 was quite similar to that of CK, …*".

Figure 5 – Information regarding floding and drying events should be included in the plots or, at least, in the legend.

Response: We added information regarding flooding and drying events in the legend (Line 308-309): "*Days 14 and 28 correspond to the flooding period, days 42 and 56 to the early drained period, and days 70 and 84 to the late drained period, respectively*".

L297 – Where are the results referring to the variable "nosZI"?

Response: The abundance of *nosZ*I was presented in Fig.S2b. For the *nosZ* gene, we primarily emphasized *nosZ*II, as it showed greater variations across treatments and sampling times.

We revised the sentence (Line 312-313): "*In contrast, the abundance differences in nosZI among treatments (Fig. S2b) were far less pronounced than those in nosZII*".

Discussion

L308-309 – I believe that the authors are comparing their results to those presented by Xing et al. (2025). However, the sentence is not accuratelly formulated.

Response: We revised the sentence (Line 325): "*…, consistent with previous studies (Xing et al., 2025)*".

L311-312 – If the soil properties under treatment ST1 were not statistically different from those under CK, the authors should not infer that the soil properties were improved.

Response: We revised the description (Line 327-330): "*The results showed that although the soil physicochemical properties of ST1 were not significantly different from those of CK, an improving trend in soil fertility was evident from the decreased BD and increased soil nutrient contents, and this trend was supported by ST2 and ST3 treatments*".

L314 – The authors did not evaluate soil porosity or soil organic matter. Additionally, not all nutrient related variables were significantly affected by treatments.

Response: The sentence was deleted.

Conclusions

Conclusions should be more focused on the main results and their implications.

Response: We revised the Conclusions (Line 380-387): "*In general, rice straw incorporation could improve soil nutrient supply ability for crops and simultaneously intensify GHG emissions. However, we revealed that when the straw was incorporated at a suitable amount, i.e., 50% of the local rice straw yield, GHG emissions remained at low levels that did not cause a significant increase in GWP compared to CK, and soil fertility was well maintained. The major contributors to this consequence were strongly linked to the highest nosZII copy number and the lowest nirS/nosZII ratio, resulting in a significantly lower N_2O cumulative emission than CK. The CH_4 producing ability under ST1 fluctuated around the soil CH_4 holding capacity, leading to most of the produced CH_4 being consumed by methanotrophs in soil and minimal CH_4 being emitted into the atmosphere. These findings offer scientific guidance for rational straw use, sustainable rice farming, and eco-friendly paddy management*".

L365 – This sentence is too vague.

Response: The sentence was deleted.