

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 CH₄, CO₂, and N₂O 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. CH₄ 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 N₂O 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). N₂O reduction is typically mediated by two distinct clades of N₂O 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 CH₄ production, its effects on CH₄ oxidation and N₂O 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 N₂O-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 ($\alpha = 0.05, 0.01, \text{ or } 0.001$)*”.

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”*.