

## List of responses

Dear Editor and Reviewers:

Thank you for the reviewers' comments concerning our manuscript entitled "Moderate N fertilizer reduction with straw return modulates ecosystem services and microbial traits in a meadow soil" (Manuscript ID No. egosphere-2023-2498). These comments were all valuable for improving our manuscript and provided important guidance for our research. We have studied the comments carefully and have made corrections that we hope will meet with your approval. The main corrections in the paper and the responses to the reviewer's comments are as follows:

I think this is an interesting study and I carefully completed the review. The authors investigated the effects of N input levels on microbial community and agroecosystem services after straw return. And they tried to explain the relationship between N input and ecosystem services from two aspects: microbial genes abundance and module communities. This work can be accepted with the following modifications.

1. As straw was added in all the treatments in this research, the title "Moderate N fertilizer reduction with straw return modulates ecosystem services and microbial traits in a meadow soil" might be more proper.

**Reply:** Thank you for the comment. The title has been changed to "Moderate N fertilizer reduction with straw return modulates ecosystem services and microbial traits in a meadow soil". (Lines 1-2)

2. Abstract: It is recommended to add primary data support rather than purely verbal descriptions. And provide the results about other treatments to compare with 0.75N+PK.

**Reply:** Thank you for the comment. We have added specific descriptions of these data.

Specifically, no significant differences were observed in SOC, total N and P contents, straw C and N release amounts, microbial biomass C and N contents, or cellulase and

N-acetyl-D-glucosaminidase activities relative to those of 0.5N+PK and PK. Greenhouse gas mineralization was reduced with decreasing N input. Moreover, 0.75N+PK had the highest straw biomass and yield, which were significantly higher than those in 0.5N+PK and PK. (Lines 34-38)

3. L29-30 If possible, “N+PK” and “PK” can be changed as “1N+PK” and “0N+PK”, which is more comparable and clear to express the significance of different treatments.

**Reply:** Thank you for the comment. In my opinion, the current version is clear enough and can be clearly distinguished from 0.9N+PK and 0.8N+PK.

4. In the introduction, please replenish relative introduction on reduction of N fertilization.

**Reply:** Thank you for the comment. We have added the necessary descriptions.

Recent research has indicated that an appropriate reduction in N fertilizer input can not only maintain crop yield by increasing N fertilizer use efficiency but also promote soil health by regulating the soil C:N ratio (Chen et al., 2014). However, an excessive reduction in N fertilizer input can lead to an “N-mining” effect, resulting in the loss of soil organic matter, which reduces crop yields (Chen et al., 2014). (Lines 58-62)

5. Line 33: “..... N deficiency.” should be “ ..... reduction of N fertiliser application .”

**Reply:** Thank you for the comment. We have revised this sentence.

The 0.75N+PK treatment had overall positive effects on soil fertility, productivity, straw decomposition, and microbial abundance and function and alleviated greenhouse gas emissions. (Lines 32-34)

6. Materials and methods, Supply references for all the determination methods.

**Reply:** Thank you for the comment. We have added the appropriate references to the Materials and methods.

7. In the results, the description of “ with the increase of N fertiliser application” should be “with the reduction of N fertiliser application”, and redescribe relative results”

**Reply:** Thank you for this comment. We have rewritten this sentence.

Regarding greenhouse gas emissions, with decreasing N fertilizer application levels, CO<sub>2</sub> and N<sub>2</sub>O emissions gradually decreased. (Lines 308-309)

8. What does mean of “straw biomass” in Fig4?

**Reply:** Thank you for this comment. “Straw biomass” refers to the dry weight of aboveground straw, which is explained in the Materials and methods. (Lines 184-185)

9. Lines 73-82: This paragraph is intended to express the role of microorganisms in ecosystem services, but needs to complement the examples of previous research.

**Reply:** Thank you for the comment. We have added some examples.

For example, Ning et al. (2020) found that long-term manure application increased the abundance of specific fungi involved in yield improvement. Duan et al. (2021) reported that adequate N input improved the cellulose degradation ability of bacteria. (Lines 79-81)

10. Line 112: It is suggested to add the purpose of this study.

**Reply:** Thank you for the comment. We have added the purpose.

The purpose of this study was to optimize the N fertilizer application rate to achieve soil ecosystem multifunctionality and explore the potential microbial mechanism in a Mollisol. (Lines 101-103)

11. Line 297: Add a related version of the R language.

**Reply:** Thank you for the comment. We have added the version of the R language used.

12. In discussion, I recommend supplementing the evidence on the relationship between straw C and N release and greenhouse gases. As we all know, the efficient conversion of straw C and N into SOM rather than CO<sub>2</sub> and N<sub>2</sub>O is a key issue. Adding relevant content can greatly improve the quality of your manuscript.

**Reply:** Thank you for the comment. In this study, straw C and N release and greenhouse gas emissions did not occur in the same environment. Straw C and N release levels were determined in field experiments, while greenhouse gas emissions were calculated in cultivation experiments.

In addition, the two experimental periods were not consistent; straw C and N release were measured after approximately 5 months, and greenhouse gas emissions were measured after only 60 days.

Therefore, the relationship between these parameters was not discussed in this study. Your suggestion is very insightful, and relevant experiments will be carried out in subsequent research to reveal the relationships between them.

13. L403-404 It is not clear to show the description of Fig.4.

**Reply:** Thank you for the comment. We have rewritten the description.

The heatmap showed close correlations of fertilizers (N input and straw return) with soil stoichiometry and microbial traits. (Lines 395-396)

14. Lines 454-456: It is suggested to revise this sentence.

**Reply:** Thank you for the comment. We have revised this sentence.

Higher MBC and MBN values, as well as relevant enzyme activities, were also observed under the N-rich treatments, indicating the strong positive impact of abundant N fertilizer application. (Lines 440-442)

15. Line 500: Deleting "Interestingly"

**Reply:** Thank you for the comment. We have deleted this term.

Thank you for your valuable comments. We hope our responses will meet with your approval.