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. egusphere-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:

General Review: This study investigates the impacts of different N fertilizer rates on maize through a four-year field experiment, exploring its effects on ecosystem functions such as soil fertility, straw degradation, greenhouse gas emissions, and maize yield. The authors introduce the terms "soil multifunctionality" and "multiple ecosystem services" in a small-scale experiment, when in fact these terms are used in large-scale studies, generally in several locations and with many biological replicates. Also, the manuscript introduces the term "ecosystem dis-services" to represent greenhouse gas emissions, a usage that is relatively uncommon. Furthermore, the research focusses too much on "straw return" without including a treatment group without straw return, complicating the ability to draw robust conclusions about its effects on the experiment. The study lacks essential innovation as the impact of varying N fertilizer rates on soil fertility and greenhouse gas emissions is well studied. Despite this, the manuscript provides valuable insights, such as the revelation that reducing N fertilization by 25% yields comparable results to conventional N application while simultaneously decreasing greenhouse gas emissions. Furthermore, the study highlights increased straw decomposition and N mineralization with a 25% N reduction, which was quantified through the assessment of functional genes (cbhI and GH48) associated with fungi and bacteria, respectively. Regrettably, in its current form, I cannot endorse the publication of this manuscript in SOIL. I recommend that the authors reshape the manuscript's perspective, emphasizing the robust findings, avoiding the use of "soil multifunctionality," and consider submitting a completely revised version.

Specifics comments:

The introduction section is too extensive and it should be reduced to maximum 2.5 pages with a double space.

1. The introduction section is too extensive and it should be reduced to maximum 2.5 pages with a double space.

Reply: Thank you for the comment. We have reduced the Introduction section.

2. Line 33: How could this be an N deficiency effect since the 0.75N+PK treatment had a slightly better corn yield than the N+PK treatment and the same amount of straw biomass?

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 32-33)

3. Lines 73-74: Please, explain what is an “opaque environment”.

Reply: Thank you for the comment. Originally, “opaque environment” meant "invisible to the naked eye". According to your comments above, the sentence has been deleted.

4. Lines 153-154: It's important to address the exact time that the litter bags were left in the field.

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

On May 2nd, 2021, litter bags containing straw were buried at 10 cm depth in a random design to prevent bags associated with a given decomposition stage from being placed together. The litter bags were collected after harvest on October 1st, 2021. (Lines 139-141)

5. Lines 209-211: The authors should specify the reference used to calculate the soil multifunctionality.

Reply: Thank you for the comment. We have added the reference.

6. Lines 212-213: The method description indicates that N2O and CO2 emissions were given the same weight as other soil attributes. This means that higher emissions contribute positively to soil multifunctionality. Therefore, the reader may be confused by thinking that higher greenhouse gas emissions generate beneficial effects on the environment, whereas this effect is exactly the opposite. I suggest the authors review how they work with "soil multifunctionality".

Reply: Thank you for the comment. In this study, greenhouse gas emissions had a negative effect on soil multifunctionality. We have added relevant descriptions to the Materials and methods.

Notably, the opposite numbers of greenhouse gas emissions were used to evaluate their negative effects. (Lines 198-199)

7. Line 218: What is a success of DNA extraction? The agarose gel in a electrophoresis can evaluate the DNA integrity, i.e., if the DNA is fragmented or not.

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

The quality of the extracted DNA was characterized by electrophoresis on 1% (wt/vol) agarose gel. (Lines 206-207)

8. Lines 227-228, 238-239: The PCR stages should be addressed correctly as stage of DNA denaturation, repeated cycles of DNA annealing and the final stage of extension.

Reply: Thank you for the comment. We have revised these sentences.

The thermal qPCR profiles for bacteria and fungi were as follows: 95 °C for 2 min for DNA denaturation, 35 cycles of (95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and 80 °C for 15 s) for DNA annealing, and 81 °C for 10 s for DNA extension; and 95 °C for 10 min for DNA denaturation, 40 cycles of (95 °C for 15 s, 52 °C for 30 s, 72 °C for 30 s, and 79 °C for 30 s) for DNA annealing, and 81 °C for 10 s for DNA extension, respectively. (Lines 214-218)

The qPCR thermal profiles for the target genes of *GH48* and *cbhI* were as follows: 95 °C for 5 min for DNA denaturation, 40 × (94 °C for 30 s, 60 °C for 45 s, and 72 °C for 90 s) for DNA annealing, and 84 °C for 10 s for DNA extension; and 94 °C for 4 min for DNA denaturation, 40 × (94 °C for 45 s, 50 °C for 30 s, and 72 °C for 60 s) for DNA annealing, and 81 °C for 10 s for DNA extension, respectively. (Lines 226-230)

9. Lines 282-283: There are no RDA results in the main manuscript or in the supplementary material.

Reply: Thank you for the comment. We have deleted the mention of RDA.

10. Lines 294-295: The heatmap was based on a correlation method? Which one?

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

A first heatmap was constructed to reveal the associations between soil ecosystem services and microbial module communities. Another heatmap was constructed to reveal the associations between microbial traits and fertilizers, soil properties, greenhouse emissions and ecosystem multifunctionality. (Lines 286-288)

11. Lines 309-310: Please explain which microbial function. Also, the microbial biomass was sensitive for what? Microbial biomass was increased or reduced? What are the magnitude changes of your treatments?

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

The MBC and MBN contents, as well as the associated enzyme activities, changed after the application of different N fertilizer rates. (Lines 302-303)

12. Lines 315 and every time that the term “ecosystem dis-services” is used: I suggest the authors to use the correct terminology of "greenhouse gas emissions" instead of "ecosystem dis-services". This can prevent readers from making wrong associations with the authors' results and increase the manuscript visibility for readers looking for more information on N2O emissions under different rates of nitrogen fertilizer use.

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

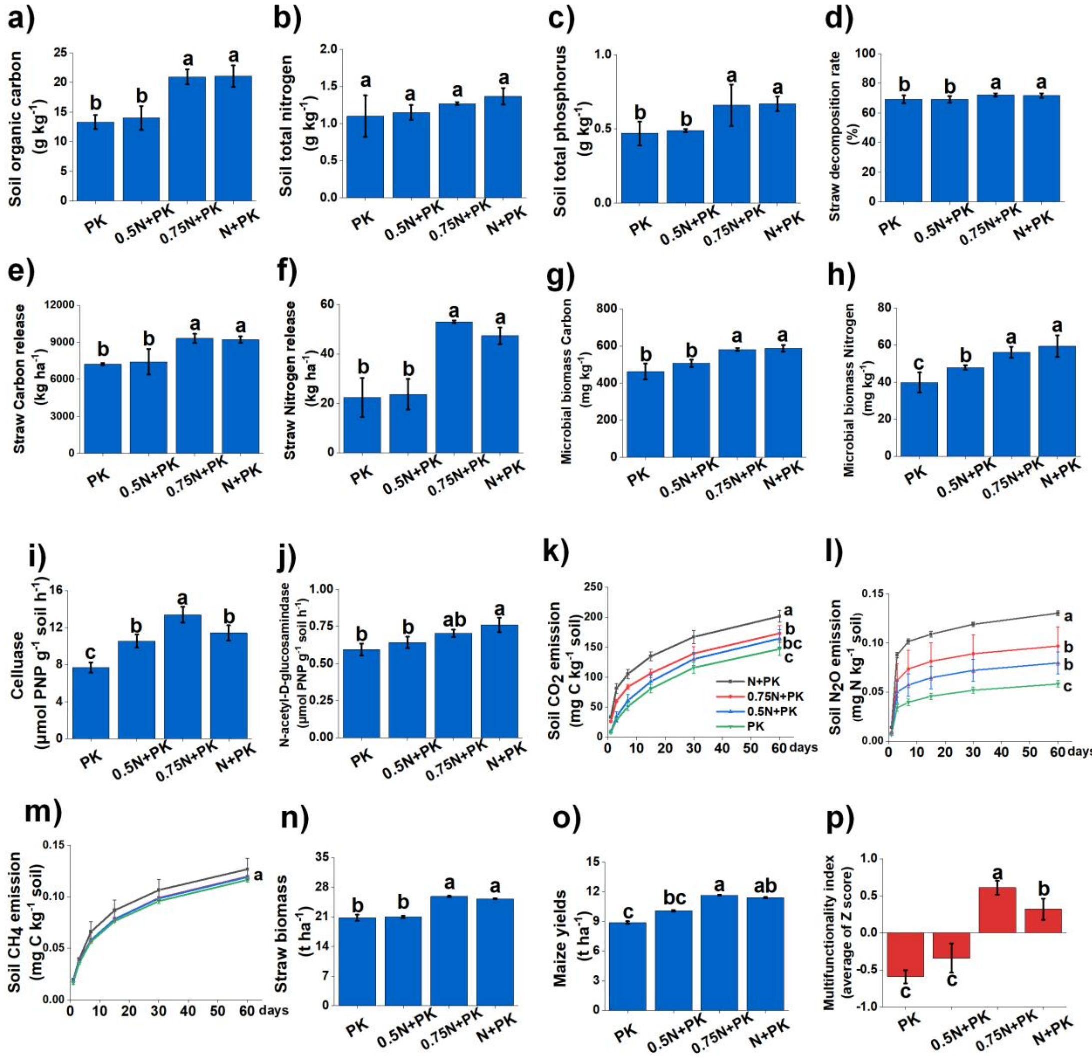
Regarding greenhouse gas emissions, with decreasing N fertilizer application levels, CO_2 and N_2O emissions gradually decreased. (Lines 308-309)

13. Lines 319-21: This should be moved to the discussion section.

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

14. Line 332: Figures 1K, 1L, and 1M use the term "mineralization" whereas it should use the term "emission". I suggest the authors change it to avoid the reader's misunderstanding.

Reply: Thank you for the comment. We have revised the terms in Figure 1.



15. Line 367 and Figure S3 (supplementary material): The ANOSIM test was used in the NMDS plot and it should be included in the Material and Methods section.

Reply: Thank you for the comment. We have added the necessary content to the Materials and Methods.

Analysis of similarities (ANOSIM) was used to examine the significant differences in microbial community structure under different fertilization treatments. (Lines 273-275)

16. Lines 372-373: The multitrophic network seems to be an important result of the authors' co-occurrence analysis. Therefore, it should be included in the Material and Methods section.

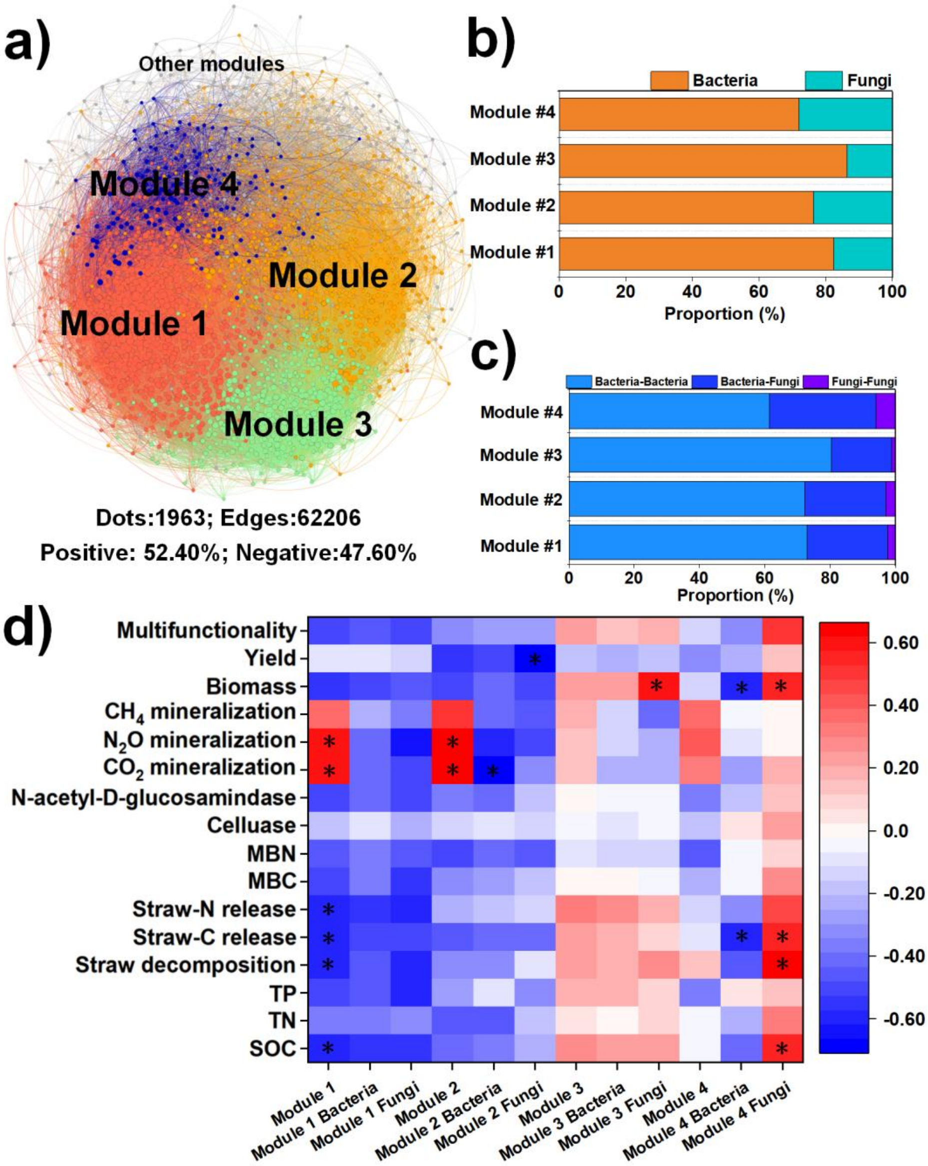
Reply: Thank you for the comment. We have added the necessary content to the

Materials and Methods.

Network visualization of microbial taxa and ecological clusters of microbial phylotypes was conducted with Gephi software. (Lines 278-280)

17. Line 381: Please include the network topological properties to have a better overview of the number of nodes, edges and the proportion of positive and negative edges.

Reply: Thank you for the comment. We have added these properties.



18. Lines 416 and 419: Figure 3D shows the contribution of fungal OTUs to the soil multifunctionality index. Perhaps the authors made a mistake and referenced the

wrong figure. I suggest a review of this part of the results.

Reply: Thank you for the comment. We have corrected these faults.

Moreover, to clarify the potential main drivers of soil ecosystem services, the correlations between microbial physiological traits and soil properties were calculated to determine the role of the microbial community in soil ecosystem multifunctionality (Fig. 2d). The results indicated that the microbial module community was significantly correlated with soil ecosystem services. The communities of modules 1 and 2 and the fungal community in module 4 showed potential contributions to soil ecosystem services (Fig. 2d). (Lines 405-410)

19. Lines 433-434: Again, the figure reference is incorrect. I think this result may refer to Figure 3C and 3D. I suggest the authors review whether all results are addressed to their respective figures.

Reply: Thank you for the comment. We have corrected these faults.

We selected the 20 keystone taxa at the species level (10 bacterial and 10 fungal taxa) with the highest relative abundance for further analysis. The random forest model results indicated that specific keystone taxa strongly influenced soil ecosystem multifunctionality (Fig. 3c and d). (Lines 422-424)

20. Lines 451-492: Weak discussion on the effects of reducing the rates of N application. The discussion is focused too much on N application and not on different N rates.

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

Our results indicated that 0.75N+PK maintained parameters related to the soil fertility index and net primary production compared to N+PK. The results demonstrate that the effects of 0.75N+PK on soil ecosystem services are similar to those of N+PK. Therefore, it can be concluded that 0.75N+PK is a more efficient and effective option for improving soil ecosystem services. Moreover, 0.75N+PK may enhance N fertilizer use efficiency and stimulate microbial functioning by altering the stoichiometry of C, N and P in the soil, ultimately promoting soil fertility and crop yield (Liu et al., 2010).

Reducing the amount of N fertilizer by more than 50% led to insufficient N input to meet the needs of both crops and microbes, resulting in a decline in soil health (Williams et al., 2013). (Lines 446-454)

21. Lines 451-453: This sentence is confusing. The variables soil fertility", "C and N release" and "crop productivity" were defined as ecosystem services by the authors. How are these variables themselves are contributing to the increase in ecosystem services?

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

Soil fertility, straw decomposition, C and N release amounts, and crop productivity were mostly higher under 0.75N+PK and N+PK than under the other treatments, implying that better soil multifunctionality was achieved. (Lines 438-440)

22. Lines 473-474: It is important to describe what are the "unintended consequences".

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

However, excess N input also causes increased greenhouse gas emissions. (Line 467)

23. Line 493: There is no data from microbial composition (i.e., taxonomy) in this manuscript. It's better to change the title to "microbial abundance".

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

4.2 Responses of microbial abundance and function to straw return with N fertilizer reduction (Line 489)

24. Lines 502-505: What was the C:N ratio on the referenced paper? It's similar to the authors' results? It's crucial to compare the results from the authors with similar results found in the literature.

Reply: Thank you for the comment.

Gao et al. (2015) indicated that the optimal ratio of C to N inputs was 20:1, which may meet the demands of maize growth and microbial proliferation. (Lines 500-502) Therefore, the 0.75N+PK treatment with a higher C:N ratio (16.47) may facilitate the proliferation of microorganisms and promote an increase in microbial abundance. (Lines 509-510)

25. Line 506: I was expecting some discussion about the fungi:bacteria ratio since the 0.75N+PK treatment showed a better result than the N+PK treatment.

Reply: Thank you for the comment.

Gao et al. (2015) indicated that the optimal ratio of C to N inputs was 20:1, which may meet the demands of maize growth and microbial proliferation. It is well known that fungi have a stronger C utilization efficiency than bacteria (Duan et al., 2021). Therefore, increasing fungal abundance and lowering the ratio of bacteria to fungi are crucial for straw degradation and SOC accumulation. Previous studies have shown that the C:N ratio of fungi is greater than 20; however, the C:N ratio of bacteria is less than 10. Excessive N fertilizer input may reduce the soil C:N ratio, while low N fertilizer input cannot meet the growth requirements of crops and microorganisms (Ning et al., 2020). Therefore, appropriate enhancement of the soil C:N ratio can increase the ratio of fungi to bacteria, stimulate fungal function, and promote straw degradation and SOC accumulation. (Lines 500-509)

26. Lines 516-517: What would be an "adequate N fertilizer"? It is better to inform that the 0.75N+PK treatment showed better results than the N+PK treatment. Moreover, the term "multiple ecosystem services" is not the most appropriate here since both the cbhI and GH48 genes are related to the straw degradation function.

Reply: Thank you for the comment. We have revised these sentences.

Our results indicated that 75%-100% N fertilizer could upregulate fungal and *cbhI* gene abundances, which may lead to straw decomposition and SOC accumulation. It is therefore necessary to further explore the potential associations between microbial traits and ecosystem services under varying N fertilizer input levels. (Lines 520-523)

27. Lines 534-535: It's better to discuss your main results and not the methods used in data analysis.

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

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