List of responses

Dear Editor

Thank you for your comments concerning our manuscript entitled "**Moderate N fertilizer reduction with straw return modulates cropland functions and microbial traits in a meadow soil**" (Manuscript ID No. egusphere-2023-2498). Those comments are all valuable and hopeful for revising and improving our manuscript, as well as the important guiding significance to our research. We have studied comments carefully and have made the correction which we hope meet the approval. The main corrections in the paper and the responses to the editor comments are as following:

1. L42 & 43 - causality not appropriate, these are correlations

Reply: Thanks for your comments. The sentences have been revised.

The *Lasiosphaeriaceae* within module 1 community showed significant positive correlations with straw degradation rate, C and N release, while the *Terrimonas* within module 3 community showed a significant positive correlation with production, which were conducive to soil multifunctionality. (Lines 41-44)

2. L99 - introduction of the word 'traits' here requires support before - for instance I suggest inserting the word as an equivalent to the properties and function, in the paragraph (L85-95) discussing those traits.

Reply: Thanks for your comments. The sentences have been revised.

Microorganisms contribute to soil functions by modulating microbial traits (e.g., function, community composition and succession), which are influenced by different N fertilizer input levels (Bradford et al., 2014; Chen et al., 2019a). (Lines 87-89)

3. L100 - Similarly, the uncommon term 'ecosystem disservices' should be introduced earlier (e.g., L69)

Reply: Thanks for your comments. The necessary content have been added.

However, crop fields suffering from superabundant exogenous materials may result in ecosystem negative effects. For example, excess organic materials usually have low reutilization efficiency

(Hou et al., 2020); the majority of N in straw is released into the atmosphere as oxynitride, such as nitrous oxide (N_2O) (Wang et al., 2019; Sun et al., 2021); Subsequent literature highlighted that straw return significantly elevates greenhouse gas emissions so that less than 15% of straw-derived N can be transformed into soil and become SOM (Yin et al., 2018; Wu et al., 2019). Moreover, as you suggested later, we have replaced ecosystem disservices by ecosystem negative effects. (Lines 68-73)

4. L114-117 - rename the treatments OR clarify that the only the urea fertilizer is being referred to with the titles '0.75N, 0.5N, or 0N', because these titles do not account for the N in DAP that was added to all plots (DAP is 18% N, or 45 kg N h-1 y-1)

Reply: Thanks for your comments. Due to my negligence, the original text incorrectly described the addition form of phosphate fertilizer. In this study, calcium triple superphosphate is the only phosphorus source in the soil rather than diammonium phosphate. The necessary explanation have been added and the incorrect description has been corrected.

Four treatments received different N fertilizer input levels after straw return to the field for 4 years as follows: (1) regular chemical fertilization, N+PK (300 kg urea (N 46%) ha^{-1} yr^{-1}, 250 kg calcium triple superphosphate (P_2O_5 46%) ha^{-1} yr^{-1}, 150 kg potassium chloride (K_2O 50%) ha^{-1} yr^{-1}); (2) 25% reduction of N fertilizer, 0.75N+PK (225 kg urea ha^{-1} yr^{-1}, 250 kg calcium triple superphosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}); (3) 50% reduction of N fertilizer, 0.50N+PK (150 kg urea ha^{-1} yr^{-1}, 250 kg calcium triple superphosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}); and (4) no N fertilizer, PK (250 kg calcium triple superphosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}). Urea is the only nitrogen source in the soil. (Lines 112-120)

5. L286 - please describe how the heatmaps were constructed (software, assumptions)

Reply: Thanks for your comments. The necessary content have been added.

To construct the relationship between fertilization, soil function, and microbial traits, two heatmaps were constructed in this study (Origin 2022). The first heatmap was constructed to reveal the associations between cropland properties with microbial module comminuties. And another heatmap was constructed to reveal the associations between microbial traits and fertilizers, soil properties, greenhouse emissions and ecosystem multifunctionality. (Lines 288-292)

6. L295 - replace 'ecosystem services' with appropriate term (e.g., cropland properties, cropland traits), because these are not ecosystem services (ES). ES are services that are supporting (water infiltration, nutrient cycling, C sequestration), regulatory, provisioning, or cultural, provided by the environment, that directly or indirectly support humans. The functions described in this manuscript contribute to supporting ES, but are not in themselves ES. Also, that is a different topic than multifunctionality. Throwing around these terms will frutrate readers.

Reply: Thanks for your comments. In the original paper, we took soil fertility, microbial function, crop yield, etc., as indicators of ecosystem services, and greenhouse gas emissions as indicators of ecological dis-services. As you suggest, there are limitations to expressing these properties in terms of ecosystem services. After careful consideration, we have decided to replace all descriptions of "ecosystem services" with "cropland properties", "cropland traits" and "soil functions". Moreover, "ecosystem dis-services" was replaced by "ecosystem negative effects".

The relevant sentences have been revised.

7. L309 - Fig 1k, 1l (not 1m)

Reply: Thanks for your comments. The sentence has been revised.

For greenhouse gas emissions, with the decrease in N fertilizer application levels, CO_2 and N_2O emissions gradually decreased (Fig. 1k, l). (Lines 311-312)

8. L310 - Fig 1m (not 1l)

Reply: Thanks for your comments. The sentence has been revised.

No significant difference was observed in CH_4 emissions under the different fertilization treatments (Fig. 1m). (Lines 312-313)

9. L311 - remove 'as expected' as this level of detail was not provided in the hypotheses (or change hypotheses)

Reply: Thanks for your comments. We have deleted 'as expected'.

The 0.75N+PK treatment achieved the highest multifunctionality index (0.61), followed by N+PK (0.32), 0.5N+PK (-0.34) and PK (-0.59) (Fig. 1p). (Lines 314-315)

10. Fig 1n: please change 'straw biomass' to 'AG biomass' to be consistent. Easy to confuse with the straw litter bag assay.

Reply: Thanks for your comments. We have revised this figure.

And you can see these in Fig.1.

11. Fig 2d: replace 'mineralization' with 'emissions' or 'flux rate' (also l.411)

Reply: Thanks for your comments. We have revised these figures.

And you can see these in Fig.1 and Fig.2.

12. L395 - the text here and in the fig 4 caption should say '…close correlations of N input (fertilizer and straw return)…'

Reply: Thanks for your comments. The sentence has been revised.

The heatmap showed the close correlations of N input (fertilizer and straw return) with soil stoichiometry and microbial traits (Fig. 4). (Lines 396-397)

13. L402 &L404 - replace 'affecting' with 'correlated to'

Reply: Thanks for your comments. The sentence has been revised.

The results indicated that the N input level, straw biomass and soil C:N ratio were the most prominent abiotic factors correlated to the ecosystem multifunctionality index, while some biotic factors, such as the abundance of genes encoding cellulose-degrading enzymes, significantly correlated to the ecosystem multifunctionality index. (Lines 402-405)

14. L424-426 - why aren't these OTUs assigned taxonomy here?

Reply: Thanks for your comments. We have added the relevant content.

Bacterial *Terrimonas* (in module 1), Myxococcales (in module 2) and *Terrimonas* (in module 3) were highlighted as essential predictors of soil ecosystem multifunctionality, and fungal Lasiosphaeriaceae (module 3) was also found to be an important variable for predicting its changes. (Lines 426-408)

15. L505 - fungal C:N is typically less than 20, for instance 15.7:1 (250:16:1 for C:N:P) with a wide range (https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2017.01281/full). Perhaps you could provide a reference to support the value of 20:1?

Reply: Thanks for your comments. We have revised these inappropriate descriptions.

Previous studies have shown that C:N:P ratio of soil microbial biomass was stable at 60:7:1 (Cleveland et al., 2007) and fungal biomass C:N ratio was higher than this ratio (nearly 15.7:1) (Zhang et al., 2017). Generally, Bahram et al. (2018) concluded that higher C:N ratio may promote fungal abundance and decrease bacteria:fungi ratio. (Lines 505-508)

Reference:

Bahram, M., Hildebrand, F., Forslund, S.K., Jennifer L. A., Nadejda, A. S., Nadejda, A. S., Johan, B., Sten, A., Luis, P. C., Helery. H., Jaime. H., Marnix, H. M., Mia, R. M., Sunil, M., Pål, A. O., Mari, P., Sergei, P., Shinichi, S., Martin, R., Leho, T., Peer, B.: Structure and function of the global topsoil microbiome, Nature 560, 233 – 237, doi: https://doi.org/10.1038/s41586-018-0386-6, 2018.

Cleveland, C.C., Liptzin, D.: C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass?, Biogeochemistry 85, 235–252, doi: https://doi.org/10.1007/s10533-007-9132-0, 2007.

Zhang, J., Elser, J.J.: Carbon:Nitrogen:Phosphorus Stoichiometry in Fungi: A Meta-Analysis. Front. Microbiol. 8:1281. doi: https://doi.org/10.3389/fmicb.2017.01281, 2017.

16. L524, 525, 537. Please replace the term 'ecosystem services'. These are ecosystem or soil functions, but not ecosystem services.

Reply: Thanks for your comments. We have revised these sentences.

As we described earlier, ecosystem services have been replaced in full.

It is therefore necessary to further explore the potential associations between microbial traits and soil functions under diverse N fertilizer input levels. (Lines 525-527)

Numerous studies have shown that core microbiota play a vital role in maintaining the stability of soil microbial function and the complexity of microbial networks and then promoting soil nutrient cycling and other soil functions; (Lines 540-542)

In addition, we have also replace 'ecosystem services' with appropriate term in the whole manuscript.

17. L540 & L550 - link to the OTU number used as a label in the results, to be consistent (i.e., see remark for L 424-426).

Reply: Thanks for your comments. We have revised these sentences before. The OTU number and taxonomy are consistent.

Specific thanks to you for your insightful comments

We appreciated for Editor’s warm work earnestly and hope that the correction will meet with approval.