Response to the Comments of Reviewers

I carefully read your manuscript titled "Differential vulnerability of mineral associated and particulate organic carbon to nitrogen addition in a subtropical forest" and hereby I provide a review in the hope that it can be informative and useful. The paper describes the methods employed and presents the outcomes of a nitrogen fertilization experiment in Southern China. The main objective was to test two hypotheses that were based on previous findings. (i) N addition promotes plant biomass and can promote particulate organic carbon accumulation; and (ii) the treatment can result in increased leaching of cations which in its turn can result in lower mineral associated organic carbon. The hypotheses appear to lack objectivity and novelty, based on existing literature. For example, it is a well-known fact that nitrogen is a limiting factor to plant productivity in a considerable fraction of land, and thus, it is expected that nitrogen addition will result in biomass increase. Despite the good presentation of the results and the clear discussion, the experimental design limits the generalization power of the relationships between SOC persistence and vulnerability to N addition. Moreover, the method section is incomplete. Details about the experimental design that are important to the understanding of the research are missing. In the following points the main shortcomings of the manuscript, in my view, will be outlined.

Response: Thank you very much for your kind work and constructive comments and suggestions, which greatly improved the quality of the manuscript. Among major changes in the revised manuscript, (1) we have rewritten the hypothesis. "*We hypothesized that 1) N addition promotes POC accumulation, as the N availability induced by N addition increases plant biomass and litter input, and soil acidification leads to a decrease in microbial biomass and enzyme activity; 2) N addition decreases MAOC content, because N addition causes the depletion of exchangeable cations and attenuates mineral protection*" (Lines 102–106). (2) Based on the comments of you and Reviewer 1, we have removed the SOC persistence, and changed the title from "*Differential vulnerability of mineral-associated and particulate soil organic carbon to nitrogen addition in a subtropical forest*" to "*Nitrogen addition promotes the accumulation of soil particulate organic carbon in a* *subtropical forest*". (3) We have added detail description of experimental design in the "Study site" section. Please see Lines 110–135.

Lack of explanation for central ideas

What is SOC bridging? How do exchangeable cations affect SOC adsorption? These seems to be important mechanisms to understand the proposed trade-off. A more precise explanation is important for the understanding of the manuscript. Also, the authors could improve the discussion by explaining how the findings described in the manuscript can be useful for ecosystem modelling as stated in the abstract.

Response: Here, using "SOC bridging" may be a non-standard term, more accurately it should be an "cation bridging". Cation bridging allows for the interaction of two negatively charged surfaces such as a phyllosilicate and an organic anion. SOC can be stabilized through sorptive interactions. These interactions include sorption to minerals, like phyllosilicate clays, Al-, Fe-, Mn-oxides, poorly crystalline minerals, or polyvalent cations forming bridges to mineral or other organic soil constituents (Rowley et al., 2018). We have provided explanations in the new manuscript. Please see Lines 88–93, 294–298. In addition, "*the response of different SOC functional fractions to N addition is inconsistent. However, current ecosystem models mainly consider the bulk soil. In the future, incorporating different SOC functional fractions into ecosystem models will help more accurately predict SOC dynamics under climate change (Abramoff et al., 2022)*" (Lines 322–326). We have included this in the discussion to align it with the abstract.

Study site and methods presentation.

The description of the study area is very simplistic. It would be important for the manuscript robustness if information about the climate (e.g. annual precipitation and temperature) were present. Information about the historical land use of the area is also important.

Response: We agree with your opinions. We have added detail description of experimental design in the "Study site" section. A *Castanopsis fabri* natural forest in Daiyun Mountain National Nature Reserve in southern China's has been selected as an experimental area. The study site has a typical subtropical oceanic monsoon climate. The reserve's average annual temperature and rainfall are roughly 17.6 °C and 1850 mm, respectively, and precipitation mainly occurs during March–September. Since the establishment of the national nature reserve, this forest has not been disturbed by human activities. At the beginning of the experiment, the tree height was 15-20 m, the diameter at breast height was 20-40 cm, and the closure was about 75%. Please see Lines 110–120.

Regarding the experimental design:

1 – the base rate of N deposition is mentioned but no value is provided.

Response: Based on previous reports on atmospheric N deposition rates in the study and nearby areas (Zhou and Yan, 2001; Yuan et al., 2016), we set up the following experimental treatments: control (CT, +0 kg N ha^{-1} yr^{-1}), low-N addition (LN, +40 kg N ha^{-1} yr^{-1}), and high-N addition (HN, +80 kg N ha^{-1} yr^{-1}). Please see Lines 124–128.

2 - The randomization procedure of the plots is omitted, leaving the reader with no clue of how pseudo replication was avoided.

Response: We have added a description of the experimental design to avoid misunderstandings for readers. Please see Lines 123–135. "*In December 2019, a total of twelve 10 m × 10 m plots were established. Spacing between plots was > 10 m to avoid N fertilizer transfer. There was no significant difference in soil organic carbon (SOC), total N, and pH among different plots before N addition. The experiment adopted a completely random design, and the three N addition treatments were randomly distributed in twelve plots with four replicates per treatment".*

3 - The frequency of nitrogen additions and the concentration of the solution used are also omitted, with the authors simply stating the urea solution in deionized was "consistently sprayed".

Response: *"Starting in early May 2020, N fertilizer was added once a month during March to September. A specific amount of urea ($CO(NH_2)_2$; LN, 24.49 g; HN, 48.98 g) was dissolved in 20 L deionized water and uniformly sprayed over the low-N addition and high-N addition plots using a backpack sprayer.*

For the control plots, the same volume of deionized water was sprayed". Please see Lines 131–135.

4 - The authors mention that they did not observe any marked differences between basic physicochemical properties of the soils of the plots before N additions. If there was a sampling campaign describing the plot conditions before the experiment, these baseline indicators should be presented and explored.

Response: There was no significant difference in soil organic carbon (SOC), total N (TN), and pH among different plots before N addition (Table R1)

Table R1 Basic properties of soil at the beginning of experiment

Index	CT	LN	HN	*P*
SOC (g kg^{-1})	53.94 ± 5.29	57.29 ± 6.42	53.31 ± 2.76	> 0.05
TN (g kg^{-1})	3.13 ± 0.11	3.08 ± 0.07	3.07 ± 0.04	> 0.05
pH	5.12 ± 0.11	5.08 ± 0.13	5.10 ± 0.10	> 0.05

CT, control; LN, low-nitrogen addition; HN, high-nitrogen addition; One-way analysis of variance was performed to determine the effects of N addition on basic soil physiochemical properties (α = 0.05). *P* represents the main effect of N addition.

5 - Should we have OC_poc where we have OC_maoc in eq. 2? I have the impression that the utility of the eq. 1 is questionable because its terms are cancelled out in equations 2 and 3. The justification and explanation of the of mass recovery (eq. 1) would improve the robustness of the manuscript.

Response: We have corrected the eq. 2. Please see Line 170.

Methodological issues.

The experimental design resulted in four observations for each treatment. At each sampled plot, five samples were aggregated to form one observation. I wonder if the analysis of variance followed by an LSD test is the optimal approach to deal with the inherent limitations of an experiment of such nature. The authors could explore, for example, the application of linear mixed models using the blocks as a random effects

variable, enabling the use of the five samples while considering pseudoreplication effects.

Response: We have added a description of the experimental design to avoid misunderstandings for readers. Please see Lines 123–135.The experiment adopted a completely random design, and the three N addition treatments were randomly distributed in twelve plots with four replicates per treatment, so LSD test is the optimal approach.

Presentation of the results.

The supplementary material is 12 lines long. I suggest adding it to the main text.

Response: Done. The data is presented in Table 1.

One last detail:

L38-42: The sentence is very long. Also, the sentence starting in line 42 is confusing. I suggest rephrasing it.

Response: Done. Please see Lines 36–41.

References

Abramoff, R. Z., Guenet, B., Zhang, H., Georgiou, K., Xu, X., Viscarra Rossel, R. A., Yuan, W., Ciais, P.: Improved global-scale predictions of soil carbon stocks with Millennial Version 2. Soil Biol. Biochem., 164, 108466, https://doi.org/10.1016/j.soilbio.2021.108466, 2022.

Rowley, M. C., Grand, S., Verrecchia, É. P.: Calcium− mediated stabilisation of soil organic carbon. Biogeochemistry, 137, 27–49. https://doi.org/10.1007/s10533-017-0410-1, 2018.

Yuan, L., Li, W., Chen, W., Zhang, J., Cai, Z.: Characteristics of nitrogen deposition in Daiyun Mountain National Nature Reserve, Environmental Science, 37, 4142–4146, https://doi.org/10.13227/j.hjkx.201605184, 2016.