Dear Editor and Reviewer,

We extend our gratitude for your dedicated time invested in reviewing our manuscript and for sharing your invaluable insights. Your detailed feedback is deeply appreciated. Your constructive comments have significantly contributed to the enhancement of our work.

According to your comments, we have thoroughly revised the manuscript. We incorporated the necessary changes and refinements throughout the revised manuscript. To facilitate your review, we have provided detailed responses to each comment in blue color below.

Once again, we sincerely acknowledge the value of your time and your expert evaluation of our work. We hope that these revisions have fortified the manuscript's lucidity, precision, and overall quality.

Best Regards,

Sun Huimin on behalf of all co-authors.

Response to RC#1

This manuscript presents interesting evidence that long-term warming and N additions reduce soil carbon stocks, with significant interactions. However, the non-significant response of soil organic matter quality to the treatments was unexpected. The study has important implications for the fate of soil C in high-altitude areas where climate change is proceeding rapidly.

We greatly appreciate your comments and constructive suggestions regarding our study. Your comments and suggestions have been meticulously reviewed and integrated into the manuscript to improve its quality. Please refer to the responses for details.

The manuscript would benefit from inclusion of hypotheses regarding the changes in the SOC structure they expect to see in response to the warming and N addition treatments.

Thanks for this great suggestion. In the revision, we have added the hypotheses.

“We hypothesized that 9-years N enrichment and warming would affect SOC stock and the chemical structure of the SOC. N enrichment below a certain threshold may favor C sequestration in the alpine grassland ecosystem but warming may result in the C loss. And added N would stimulate hydrolytic enzyme activity while warming would repress enzyme activity. Finally, we hypothesized that variation in enzyme response to

N and temperature would emerge as an important explanation for variability in the effect of added N and warming on SOC stock.”

The authors demonstrated significant interactions between the two global change treatments on many of their results, including aboveground biomass, soil pH, enzymes, C/N, SOC stocks. However, they have mostly ignored these interactive effects in reporting their results and interpreting them in the discussion section. Furthermore, they built the structural equation models without considering these interactions. Why? It seems like better insights into the multiple global change effects could be gained from establishing clear hypotheses (such as the a priori model) and then testing them with the SEM. The discussion and conclusion sections will need to be completely rewritten after considering the interacting global change effects more carefully.

This study focuses more on the changes brought about by nitrogen addition and warming on the carbon pool and its chemical molecular stability. The manuscript has been supplemented with a description of the results of the interactions after taking into account the comments of the two reviewers. And it is important given the focus of SEMs on explaining outcomes and not on identifying causative variables. The SEM model does not work well for the interactions, so to emphasize the importance of the enzyme activity we have only shown the results of nitrogen addition and warming. We hope to get your understanding.

Specific comments:

L 80-81, just give the background, remove the section that sounds like methods.

Thanks for your suggestion. The description about DRIFT has been moved to the METHOD section.

L 120-133, Site description section could be better organized – keep the plant community info together with the vegetation description. How large is the area of alpine grassland in Tibet? Give a comparison to other alpine grassland areas globally. How deep are the soils on average? Make sure to explain abbreviations like TP.

All done as suggested. The Site description section has been reorganized. Surface layer (0-10 cm) soils were collected in this study, see Experimental design section please. The description of the area of grassland ecosystem in the Tibetan Plateau has been added. The abbreviations have been explained well.

L 152, keep the verb tense consistent; past is preferred.

Done as suggested.

L 153, how was bulk density determined?

The METHOD has been added as follows. ‘Bulk density samples were dried at 105 °C for 48 h and calculated by dividing the oven-dried soil mass by the steel cylinder volume (100 cm^3) because coarse fragments (stones or large roots) were not obtained in ring samples.’

L 193, please give a brief background in the intro on the connection between the hydrophobic/hydrophilic ration and soil aggregation stability, including how it relates to SOC stability.

Done as suggested. We added more explanations: ‘The hydrophobic C/hydrophilic C (HB/HI) ratio, (C0–45 + C110–165)/(C45–110 + C165–210), was used to reflect the stability of soil aggregation (Spaccini et al. 2006, Wang et al. 2010). The higher values of HB/HI ratio indicated that SOC was more hydrophobic (Cao et al., 2016), which, in turn, implied that SOC was more stable (Spaccini et al., 2006, Wu et al., 2014).’

L 218, make sure to define all abbreviations on first use.

Done as suggested. Other parts of the text have also been scrutinized and corrected.

L 260, give year of citation

Done as suggested.

L 274 and L 325, you should not ignore the significant interaction between warming and N addition. L 282, the interaction that you observed between N and warming on AGB should also not be ignored.

Thanks for your suggestion. The content about AGB has been rewritten as follows:

‘We noticed that warming significantly reduced AGB under N1 and N2 enrichments in this study (Figure 2).’

Besides, since warming and nitrogen addition have different effects on the organic carbon pool, the effect of nitrogen addition has been added to the discussion in a separate paragraph. See the following please.

‘While N fertilization exerts both direct and indirect impacts on SOC, its influence on carbonates is direct, leading to continuous losses. This not only serves as a source of atmospheric CO2 (Kim et al., 2020; Raza et al., 2020; Zamanian et al., 2018) but also degrades soil structure and affects physical, chemical, and biological properties (Meng and Li, 2019). Under acidic conditions, this process induces fundamental changes in microbial community composition and enzyme activity critical for SOC stability (Rowley et al., 2020). In ecosystems characterized by N restriction, such as permafrost and peatland regions, N enrichment enhances N availability, accelerating the decomposition of labile organic C. This, in turn, results in decreased soil C availability

(Craine et al., 2007; Janssens et al., 2010; Song et al., 2017). A previous study at our research site revealed a significant reduction in the soil labile C pool within the particulate organic C fraction with increasing N enrichment, signifying a decline in soil C availability (Chen et al., 2019). Our findings demonstrate that N enrichment significantly stimulates extracellular enzyme (EnC) activities and enhances microbial demand for C (Figure 2), aligning with prior research indicating that added N stimulates the activity of soil cellulose-degrading enzymes (e.g., cellobiosidase (CB) and β-glucosidase (BG)) (Carreiro et al., 2000; Saiya-Cork et al., 2002; Chen et al., 2017). This stimulation may be attributed to the increase in C-acquiring enzymes resulting from heightened microbial demand for C, especially in N-limited ecosystems (Keeler et al., 2009). Previous studies suggest that N enrichment could induce C limitation by reducing plant allocation to fine root production, leading to lower C input into the soil (Treseder, 2008). Thus, we propose that factors beyond the thermal environment, such as N enrichment, can modulate soil enzymes and alter substrate availability. Moreover, these processes can mediate the strength of the soil C-climate feedback. These results underscore the importance of considering soil C availability and enzymatic activity responses, which collectively determine the response of the C balance to multiple environmental changes, for a more comprehensive understanding of C storage dynamics.'

L 355, try to write more succinctly – sentences like this contain multiple redundancies that obscure their meaning.

Done as suggested. The sentence has been rewritten in a simpler syntax to make it clearer: 'Microbial utilization of recalcitrant C pools could substantially accelerate overall soil C loss. This is because depolymerization of these recalcitrant macromolecules increases microbial accessibility to litter and SOC that was protected by recalcitrant C pools before (Schmidt et al. 2011, Lehmann and Kleber 2015, Paustian et al. 2016).'