Response to Reviewer 1

[General comment]

[**Comment 1**] This paper presents the response of three different soils (representing different altitudes) taken at two depths (0–10 and 70–80 cm) to four different kinds of freeze-thaw cycles (FTCs): long freeze/long thaw, long freeze/short thaw, short freeze/long thaw and short freeze/short thaw. The study, including a detailed experimental part and rigorous statistical analyses, highlights the differences in SOC mineralization across the altitudes, depths, and FTC types. Specifically, more frequent FTCs lead to higher SOC mineralization. Further investigation was conducted to determine the predictors of SOC mineralization. Dissolved organic carbon appears to be the most important of these predictors, both in topsoil and subsoil.

Response: We appreciate these comments.

I found the article very interesting: the experimental design, while presenting limits, is both simple and efficient, and the results and conclusions are overall well presented. However, I got a bit lost in the statistical analysis part. This will certainly be useful for statistics enthusiasts, but it is a bit difficult to follow for the average reader (which I am). Be careful not to lose us in the way, because the paper is of great interest for everyone and the findings must be clear even for those who don't master fully how you obtain them.

Response: Thank the review of the encouraging comments and pointing out the issue relevant to statistics. We have carefully refined the relevant descriptions to make them clearer, more concise and accurate. Please refer to our point-by-point responses below.

Overall, this paper is a nice addition to the knowledge on the effects of climate change – and specifically frequent FTCs – on soils and SOC mineralization.

Response: We appreciate these comments. Taking into account the suggested improvements, the manuscript has been carefully revised accordingly.

[Specific comments]

[**Comment 2**] L55: please specify the depth you consider for subsoil.

Response: Thank the reviewer for the careful review. Specified as requested. That is, the topsoil refers to 0–10 cm depth layer, while the subsoil refers to 70–80 cm depth layer.

[**Comment 3**] L65: please define 'labile' (in terms of residence time for instance). Also, you could use a reference to establish DOC as a labile pool (while it is generally thought of as labile without more precision, several studies showed that part of it can persist for decades, see Kalbitz & Kaiser, 2008 (https://doi.org/10.1002/jpln.200700043) for example).

Response: Thank the reviewer for this helpful suggestion. We agree that the term "labile" requires clearer definition, particularly given the heterogeneity of DOC pools.

We clarify that DOC represents a continuum of compounds with varying persistence, ranging from highly bioavailable substrates to more persistent fractions. To better contextualize this, we have added the suggested reference (Kalbitz & Kaiser, 2008) to acknowledge that a portion of DOC can persist over longer timescales due to physicochemical protection and sorption processes. Accordingly, the text has been revised to emphasize that DOC is treated here as an operationally defined, relatively bioavailable pool that responds rapidly to freeze–thaw perturbations, while recognizing its compositional and temporal heterogeneity. We believe these changes improve both precision and conceptual clarity in the manuscript.

[**Comment 4**] L109: what do you mean by 'quality'?

Response: Thank the reviewer for this comment. SOC decomposition is essentially a microbial process. In this study, we defined soil carbon quality from the perspective of microbial utilization particularly represented by chemical composition (such as ^{13}C NMR derived functional groups of carbon substrates) and metabolic energy yield, which directly dictates the efficiency with which microbes covert carbon into biomass. We revised the corresponding sentence accordingly to improve precision and avoid ambiguity.

[**Comment 5**] L145: where does the 0.45 value come from?

Response: Thank the reviewer for the careful review. This value (i.e., 0.45) is a widely adopted conversion factor used in the conventional chloroform fumigation-extraction method to account for incomplete recovery of microbial biomass C in the extract. In the revised manuscript, we have clarified the origin of this factor and added appropriate reference (Vance et al., 1987).

[**Comment 6**] L166: what are substrates A and B? If it is not important, it may be better not to mention it.

Response: Thank the reviewer for this suggestion. We have replaced them with more general description. The corresponding changes have been made in Line 175 in the revised manuscript.

[**Comment 7**] L250: 'On the first thawing day (Fig. 3; Fig. 4), DOC concentrations varied significantly among treatments' → if I am not mistaken, this is not visible on the figures you indicate; the uppercase letters in Fig.3 for DOC are all A and do not show the first thawing day.

Response: We are grateful to the reviewer for this careful review. Indeed, we made a mistake there. We have corrected the significance letters in Fig. 3a, and revised the corresponding text in the Results section to distinguish the overall ANOVA result from the post hoc comparison result more clearly (Line256-259).

[**Comment 8**] L255: if I interpret it well, the MBC indicates that there are more microbes in the topsoil horizon than in subsoil (which is not surprising). How do you explain that enzyme activities are higher in subsoil (although not always significantly)?

Response: We appreciate the reviewer's careful review regarding the discrepancy between microbial biomass carbon (MBC) and enzyme activities. Higher enzyme

activities in deeper layers is not novel. This is relevant to lower resource availability or harsher environment in the subsoil, and microbes are forced to be more efficient and produce higher concentrations of enzymes to mine nutrient and/or energy.

Previous studies (e.g., Schnecker et al. (2015)) (https://doi.org/10.1016/j.soilbio.2015.01.016) have shown similar patterns. For example, Fierer and Jackson (2006) (https://doi.org/10.1073/pnas.0507535103) observed that, although microbial biomass is lower in the subsoil, enzyme activity is higher due to that subsoil microbes invest more energy to depolymerize complex organic matter which is dominant in the subsoil. We have updated the text to clarify this point and provide a more detailed explanation. The corresponding changes can be found in Lines 392-396 of the revised manuscript.

[**Comment 9**] L262: it is not completely clear to me how to read the partial correlations. What do PC × DOC = 0.66 (bottom left corner) and DOC × PC = 0.79 (top right corner) correspond to? Is 0.66 the partial correlation between SOC mineralization and PC with DOC controlled, or the contrary? Sorry if this is usual for this type of graph; perhaps adding a word about it would help. Also, why aren't all the boxes filled?

Response: We appreciate the reviewer's question. In the revised manuscript, we have clarified that the first column represents zero-order correlations between SOC mineralization and each variable, without controlling for any other factors. The subsequent columns show partial correlations, where each column indicates that the variable listed on the x-axis is controlled. Accordingly, the "PC × DOC = 0.66" (bottom left) indicates the partial correlation between SOC mineralization and PC after controlling DOC. In contrast, "DOC × PC = 0.79" (top right) shows the partial correlation between SOC mineralization and DOC after controlling for PC. We have revised the text and figure legend to explicitly state this interpretation (lines 289-297 in the revised manuscript). We also clarified the structure of the matrix: blank cells indicate cases where no correlation is calculated because is being controlled in that column.

[**Comment 10**] L290: I am not familiar with path analysis, but I don't see how 'subsoil mineralization exhibited strong additional associations with C molecular composition' (that we indeed see on Fig. S7) shows on Fig.6b. All values related to the molecular structure seem quite low. Shouldn't we also see this correlation result on the path analysis?

Response: We thank the reviewer for the careful review. The reviewer is correct that in Fig. S7, subsoil mineralization exhibits strong associations with C molecular composition (e.g., AC, OAC, ACOC, HBHI, PC), while in Fig. 6b, the correlation becomes weak. This change is reasonable because the two figures present results using different approaches for addressing different questions. The path analysis in Fig. 6b include direct and indirect effects of various variables on SOC mineralization, which cannot be captured by linear correlation analyses as showed in Fig. S7. For example, molecular composition itself would be strongly regulated by climatic and edaphic conditions, which can be captured by the path analysis. So the direct effect of molecular composition becomes weak. We hope this clarifies the interpretation of the data presented.

[Technical comments]

[**Comment 11**] L31: you need spaces when using ' - ', also I think it needs a longer dash. Same later in the sentence.

Response: Thank the reviewer for the careful reading. Revised accordingly. Specifically, we used long dash ("–").

[**Comment 12**] L43: 'and are sensitive to FTCs'

Response: Corrected.

[**Comment 13**] L44: 'can recover rapidly'

Response: Corrected.

[**Comment 14**] L87: typo in 'physicochemical', and is a word missing? Perhaps 'physicochemical properties'?

Response: We appreciate the careful review. Corrected accordingly..

[**Comment 15**] L96–99: the sentence seems to be repeated.

Response: The repeated sentence has been deleted.

[**Comment 16**] L148: vertexing → vortexing?

Response: Corrected.

[**Comment 17**] L248: spaces and longer dash needed when using ' – ' .

Response: Revised accordingly.

[**Comment 18**] L343: 'due to the fact that DOC'.

Response: Corrected.

Response to Reviewer 2

[**Comment 1**] I read "Dissolved organic carbon-mediated controls dominate soil carbon mineralization in response to freeze-thaw cycles" by Jiaxin Yan et al. with great interest. The topic is timely, the experimental design is well-conceived, and the interdisciplinary approach, combining soil physics, biogeochemistry, and microbiology, is particularly valuable. I believe this work will be of broad interest to the community. However, three main concerns should be addressed before publication.

Response: Thank the reviewer for these comments. We have carefully revised the manuscript. Please refer to the point-by-point responses below.

[MAIN COMMENTS]

[**Comment 2**] Coherence between objectives, analyses, and conclusions, including statistical transparency

This is my primary concern. The stated objectives focus on how freeze-thaw cycle duration and frequency regulate SOC mineralization. However, a substantial part of the analytical framework (correlation analyses, partial correlations, path model) pools observations across all freeze-thaw treatments to identify general predictors of Rs, effectively setting aside the treatment structure. These analyses address a different question: what controls SOC mineralization in general, not specifically how freeze-thaw regimes drive it. When the discussion then uses these treatment-blind results to make mechanistic claims about freeze-thaw effects, a logical gap emerges that is not acknowledged. The analytical framework as presented does not allow the reader to clearly assess whether DOC responses differ systematically between freeze-thaw regimes.

This confusion is compounded by a lack of clarity on how temporal dependency was handled across analyses. While I am not a statistical expert, several analyses, most notably the MANOVA and the PLS-PM, include repeated measurements from the same incubation jars across multiple time points, and it is not clear how non-independence among observations was accounted for. This is particularly relevant for the PLS-PM, where all thaw-phase observations are explicitly pooled, which may inflate the effective sample size and produce overly optimistic significance levels. Reported n values also differ across figures without explicit justification, making it difficult to assess the unit of replication in each case.

The core conclusions, that FTC frequency drives cumulative mineralization and that DOC is the dominant predictor of Rs, appear robust. However, the conclusion that freeze-thaw regimes influence mineralization indirectly through DOC is difficult to evaluate as presented. The authors may well have handled these issues appropriately, but the PLS-PM as described pools repeated measurements from the same jars across time points, and it is not clear to the reader how temporal variance was separated from treatment variance in estimating the reported path coefficients. Without this clarification, it is difficult to assess whether the treatment pathways reflect genuine differences between freeze-thaw regimes or partly capture within-jar temporal dynamics. The authors should clarify how temporal dependency was handled and clearly delineate which findings speak to general soil biogeochemistry versus freeze-thaw-specific mechanisms.

Response: Thank the reviewer for this thoughtful comment. We acknowledge that part of our analytical framework pools observations across freeze–thaw treatments to identify general predictors of SOC mineralization. **These analyses were intended to address a complementary, but also critical, question: identifying controls of mineralization across all conditions.** We have explicitly revised the manuscript to distinguish between treatment effects and general controls. Importantly, we now explicitly acknowledge in the text that pooled analyses do not directly test treatment effects, and we avoid attributing causality of FTC regimes based solely on these analyses.

As mentioned by the reviewer, however, we would like to note that treatment-driven responses of Rs to FTC frequency and duration have been addressed by MANOVA and comparisons among treatments in terms of soil carbon mineralization rates, DOC, enzyme activities, etc. (e.g., the results presented Fig. 1, Table S2). **For the importance of DOC, we framed it as a mediator whose variation is subsequently linked back to FTC treatments through treatment-specific analyses** (e.g., DOC differences among regimes as reported in Fig. S3).

In terms of the temporal dependency, we have revised the analysis of PLS-PM by including measurement time as an explanatory variable in the model as time can implicitly represent accumulated or lagged effects. The updated results have been presented in Fig. 1. The results demonstrated that "Time" has significant effect on DOC.

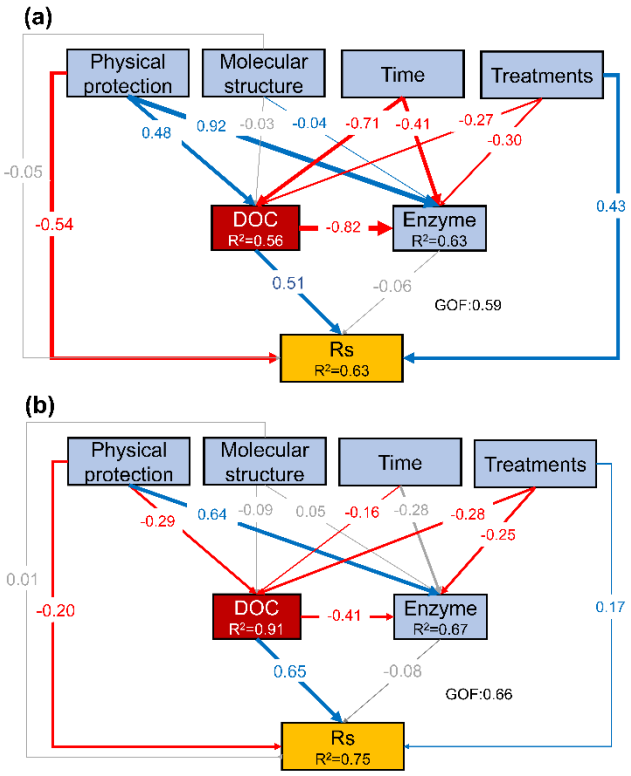

Figure 1. Path analysis results showing the direct and indirect controls of soil organic carbon (SOC) mineralization. A detailed description of the figures has been provided in the revised manuscript.

In addition, we have revised all figure captions and the Methods section to clearly define the unit of replication (e.g., incubation jar/core) and to explain differences in

sample size across analyses.

Overall, in the revision, we have (i) clearly separated treatment effects from general mechanistic controls, (ii) explicitly linked DOC dynamics back to FTC regimes, and (iii) improved transparency in statistical design and interpretation. We believe these changes substantially strengthen the coherence and rigor of the manuscript and address the reviewer's concerns.

[**Comment 3**] Enzyme activity versus enzyme concentration

The manuscript reports extracellular enzyme "activities" measured using commercial ELISA kits. ELISA assays primarily quantify enzyme protein abundance through antibody binding rather than catalytic activity. Unless the standard curve was explicitly constructed from enzymes of known catalytic activity, the use of the term "activity" throughout the text and figure legends may be misleading. Furthermore, extracellular enzymes in soils can be stabilized on mineral surfaces or organic matter, and enzymes such as β-glucosidase comprise multiple classes across diverse microbial taxa that may not be equally recognized by the antibodies used. The authors should clarify what is actually being quantified, whether any conversion to catalytic units was applied, and discuss these methodological limitations.

Response: Thank the reviewer for this important comment. We fully agree that if enzyme measurements had been conducted using antibody-based ELISA assays, the term "activity" would be misleading, as ELISA quantifies immunoreactive protein abundance rather than catalytic activity.

Upon carefully re-examining our laboratory records and assay protocols, we identified an error in the Methods description. This is largely induced by mis-communication among the lab staff. Here, we confirm that the extracellular enzymes in this study were not measured using ELISA kits. Instead, all enzymes were quantified using substrate-based microplate activity assays that directly measure catalytic activity. Therefore, the reported values represent potential extracellular enzyme activities under standardized assay conditions, rather than enzyme concentration or antibody-based estimates of protein abundance. We have revised the relevant descriptions in the revised manuscript (Lines 170–182).

[**Comment 4**] Contextualisation with in situ manipulation literature

The manuscript would benefit from a more systematic comparison with the large body of literature on in situ snow cover manipulation and soil translocation experiments, which increase freeze-thaw frequency and have documented consequences for soil carbon cycling. This literature is largely absent and would strengthen the contextualisation of the findings. In particular, the authors should discuss whether the treatment effects observed in their controlled incubation are consistent in direction and magnitude with what has been reported in field manipulation studies, and where discrepancies exist, propose possible explanations.

Response: This is a great, insightful comment. We have added a paragraph in Section 4.1 (Lines 421–433) to compare our incubation results with representative in situ snow manipulation and soil translocation studies that alter soil freeze-thaw regimes, and we also clarified this point in the discussion of limitations (Line 439–441). Overall, our

results are consistent with field studies which show that winter environmental change can affect soil carbon cycling, especially through thaw-related respiration pulses and changes in labile carbon (Buckeridge & Grogan (2010); Wu (2020)). (https://doi.org/10.1007/s10533-010-9426-5; https://doi.org/10.1016/j.catena.2020.104760) However, the magnitude of these responses are often more variable in the field and are not directly comparable with those observed in our controlled incubation. This difference is reasonable as field manipulations change not only freeze–thaw patterns, but also snow insulation, soil moisture, litter inputs, root activity, and broader site climate conditions, which may confound the impact of changes in freeze-thaw cycles.

[MINOR COMMENTS]

[**Comment 5**] L34: Does increased FTC frequency consistently increase SOC decomposition in the literature? Snow cover typically protects soil from freezing and may actually reduce decomposition. Please clarify or add appropriate references.

Response: Thank you for pointing this out. We agree that increased freeze–thaw cycle (FTC) frequency does not consistently increase SOC decomposition across the literature. Previous reviews and meta-analyses show that FTCs often increase DOC availability and can induce short-term CO_2 pulses, but the overall responses of soil respiration and C mineralization vary substantially depending on ecosystem type, soil moisture, freeze–thaw severity, and whether the evidence comes from laboratory or field studies. In snow-covered ecosystems, snow typically insulates the soil, buffers against freezing, and reduces FTC occurrence; conversely, snow removal can increase soil frost while reducing microbial biomass, exoenzyme activity, and winter soil respiration. We have therefore revised the statement in Lines 32–35 to avoid overgeneralization and to clarify that the effect of FTC frequency on SOC decomposition is context dependent rather than universally positive.

[**Comment 6**] L44: "Negative effects on microbial community composition" is vague. Please rephrase.

Response: We appreciate this comment. We have revised the sentence to: “ultimately suppress microbial activity and functioning” (Line 46-47).

[**Comment 7**] L124: Soil moisture was maintained at 60% water-holding capacity, please specify how WHC was measured or estimated, particularly for sieved soils.

Response: Specified in lines 129-134. We have clarified in the revised manuscript how soil moisture was determined. Specifically, the saturated water-holding capacity of the soil was determined using the funnel method (Shaw’s method) on fresh sieved subsamples. Briefly, 50 g of moist soil was placed in a conical filter funnel fitted with glass wool and a clamp, 50 mL of distilled water was added, and the soil was allowed to stand for 30 min. The clamp was then opened, and the drained water was collected for 30 min. The saturated water-holding capacity was calculated as the difference between the volume of water added and the volume drained, plus the water originally present in the soil. Based on this value, the incubation moisture was adjusted to 60% of the saturated water-holding capacity. During incubation, jars were weighed regularly and distilled water was added as needed to maintain the target moisture level.

[**Comment 8**] L149: Please specify centrifugation duration and relative centrifugal force.

Response: Thank the reviewer for this comment. The extracts were centrifuged at 4000 r/min for 10 min before filtration. The corresponding details have been added to the revised Methods section (Lines 149 and 153).

[**Comment 9**] Figure 1: Please add the treatment acronym (e.g. LFLT, SFST) directly as a panel title to facilitate reading.

Response: Added accordingly.

[**Comment 10**] Figure 3: If error bars reflect temporal variability within a single jar rather than biological replication, this should be stated explicitly in the caption.

Response: Thank the reviewer for this suggestion. We have revised the caption to clarify explicitly what the error bars represent. In this study, two parallel sets of incubation jars were used: one set for respiration measurements (3 elevations × 2 soil depths × 4 treatments × 3 replicates), and one set for soil property measurements (3 elevations × 2 soil layers × 4 treatments, for a total of 24 jars). For the figure 3, the three elevations correspond to three separate incubation jars for each soil depth × treatment combination. However, repeated measurements over time were not obtained from additional independent jars, but by destructive soil subsampling from the corresponding jars at each sampling time. Therefore, in figure 3, the error bars do not represent independent biological replication among multiple jars within the same treatment, but rather the variability among observations from the same treatment and soil layer across elevations and corresponding sampling times. Accordingly, for the LFLT and SFLT treatments, n = 3 represents the variability among the three elevations measured on the first day of thawing in the first freeze–thaw cycle. For the LFST and SFST treatments, n = 9 represents the variability among observations from three elevations sampled on the first day of thawing across the first to third freeze–thaw cycles (3 elevations × 3 cycles). The figure caption has been revised.