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.

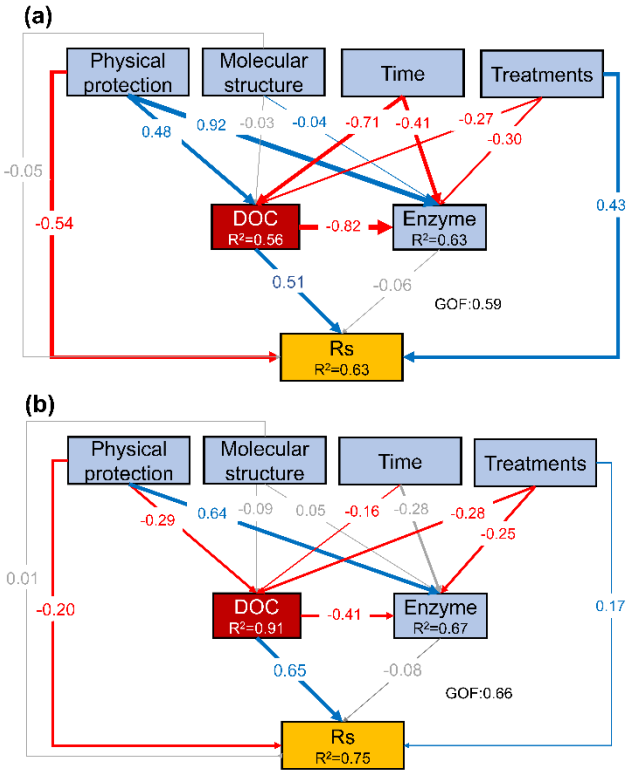

Figure 1. Path analysis results showing the direct and indirect controls of soil organic carbon (SOC) mineralization. A detailed description of the figures has been provided in the revised manuscript.

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.