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.