Dear editor

Many thanks for raising the problems and suggestions in our manuscript. The comments are helpful to the improvement of our paper, and have been incorporated into the revised version of the manuscript. Our responses to the comments are listed below:

Referee #1

1. This is an interesting and important research area and is a currently relevant study within the broad realm of soil carbon loss as a function of melting permafrost. However, I have several concerns with the study methods and therefore the interpretation of results. The very low sample size of nine for the comparisons between freezing and thaw period soils raises questions about the assertions of statistically significant differences, particularly when taking the large standard errors into account. The main concerns relate to making multiple comparisons without adjusting for those multiple comparisons that could be quelled if the data and code were provided.

Thanks for raising this meaningful question. We admit that more replicates can make our results more convincing, especially for units with strong heterogeneity like soil aggregates. However, considering the high cost of CT scanning in China, we have done our utmost to achieve a qualified sample size to ensure the credibility of the results. In total, we scanned 144 aggregates. In the future, we will try to analyze more samples if possible.

2. The second concern is the soil density fraction method, which is an outdated method from the early 1990s that has proven to be an imprecise method of density separation compared to the more commonly used sodium polytungstate method. Sodium hexametaphosphate solutions can only achieve densities of up to about 1.2-1.4 g/cm^3, whereas the commonly agreed upon

densities for separating mineral fractions are 1.6 -1.85 g/cm^3, which cannot be achieved usi ng sodium hexametaphosphate. Results relating to the density separation are therefore unreliable. I recommend removing results related to the density fractions and down-scoping this manuscript to focus on the seasonal differences in pore properties and TOC content, including the correlation table but not the RDA, which is redundant information.

We highly appreciate your insightful opinion. The density fraction method used in our study was referred from Marriott and Wander (2006), Chen et al. (2020) and Fu et al. (2023), which all used 5% (m/v) sodium hexametaphosphate. In our future studies, we will adopt your suggestion and use the sodium polytungstate method.

To reduce redundant information, the Table 2 and RDA image have been moved into Supplementary information. The scatter plots of crucial correlations replaced them to demonstrate the relationships more clearly.

References:

Chen, J., Xiao, W., Zheng, C., Zhu, B. 2020. Nitrogen addition has contrasting effects on particulate and mineral-associated soil organic carbon in a subtropical forest. Soil Biology and Biochemistry 142, 107708. doi: 10.1016/j.soilbio.2020.107708.

Fu, C., Li, Y., Zeng, L., Tu, C., Wang, X., Ma, H., Xiao, L., Christie, P., Luo, Y., 2023. Climate and mineral accretion as drivers of mineral-associated and particulate organic matter accumulation in tidal wetland soils. Global Change Biology 30, e17070. doi: 10.1111/gcb.17070.

Marriott, E.E., Wander, M.M., 2006. Total and labile organic matter in organic and conventional farming systems. Soil Science Society of America Journal 70, 950-286. doi: 10.2136/sssaj2005.0241.

3. The introduction is lengthy and could be revised to include less ancillary information and grammatical structure could be improved throughout.

Many thanks for your valuable comment. We have made careful revisions throughout the introduction.

Specific Comments

1. SOC fractionation performed according to 1992 methods using sodium hexametaphosphate. This is an outdated method that should be retired in favor of using sodium polytungstate solutions for more precise density separation. Only in cases where the researcher is building on previous data to form long-term datasets would it still be appropriate to use sodium hexametaphosphate for comparability between studies.

We highly appreciate your insightful opinion. The density fraction method used in our study was referred from Marriott and Wander (2006), Chen et al. (2020) and Fu et al. (2023), which all used 5% (m/v) sodium hexametaphosphate. For recent related studies on the QTP, the sodium hexametaphosphate was also used by Pan et al. (2024), Gu et al. (2024), etc. In our future studies, we will adopt your suggestion and use the sodium polytungstate method.

References:

Gu, J., Yang, F., Song X., Yang, S., Zhang, G., 2024. Edaphic regulation of soil organic carbon fractions in the mattic layer across the Qinghai-Tibetan Plateau. Science of the Total Environment 943, 173814. Doi: 10.1016/j.scitotenv.2024.173814.

Pan, Y., Ren, L., Huo, J., Xiang, X., Meng, D., Wang, Y., Yu, C., Liu, Y., Suo, J., Huang, Y., 2024. Soil geochemistry prevails over root functional traits in controlling soil organic carbon fractions of the alpine meadow on the Qinghai-Tibet Plateau, China. Catena 237, 107814. doi: 10.1016/j.catena.2024.107814.

2. Figure 2 is excellent!

Thank you very much!

3. Table 2 is labeled as correlations between SOC content, soil microbial characteristics. It seems that microbial characteristics is not meant to be included since none of the variables presented fit that category.

We apologize for our mistake. We have removed “soil microbial characteristics” from the title. The current title is “Correlations between SOC content and soil structure of soil aggregates in the freezing period and thawing period”.

4. Actual p-values should be provided in the text instead of presenting them as $p < 0.05$.

We highly appreciate your valuable suggestion. The actual p-values have been added in the text throughout the revised manuscript. For example, in Line 317-318, “The TOC and MAOC contents were both positively correlated with pore length density ($P=0.045$ and $P=0.006$, respectively).”

5. Fig. 5: When conducting multiple comparisons with the low sample size of nine, caution must be taken in interpretation of results. Without seeing the data itself, it is difficult to assess the validity of these results, given the high variability and low sample size. It is likely that the proportion of significant results would be relatively low given the sample size and variability. Further scrutiny of the data and statistical tests is necessary.

Thanks for reminding us this crucial issue. We certainly hoped to analyze as many samples as possible, especially for aggregates, which are highly heterogeneous structural units. However, considering the high cost of CT scanning in China, we could only meet the standard of $n=9$ to assure convincing results. We will try to expand our sample number if possible in future studies.

6. Table 2 and Fig. 7 effectively present the same information – that is the strength and direction of correlation among different covariates, so only one of the two should be presented.

Many thanks for raising this question. To avoid the data redundancy, the Table 2 has been moved into the Supplementary information.

7. The supplementary data table should include standard error for each variable measured.

We highly appreciate your comment. The standard error of variables has been added in the Supplementary Tables.

I would be happy to provide technical corrections for a revised version of the manuscript.

Thank you very much for your affirmation and valuable comments on our research.

Referee #2

General Comments

1. This manuscript presents field data of soil aggregate pore structure and carbon content through an annual freeze-thaw cycle. The measurements appear to have been carefully executed, and demonstrate some trends throughout the year for both pore structure and carbon content. The work also demonstrates strong correlations between some pore structure observations and carbon cycling through the year. Most strikingly, POC and MAOC pools strongly are associated with different pore characteristics during the freezing and thawing seasons. The review of soil aggregate FT mechanics is quite extensive.

   Many thanks for acknowledging our work.

2. Despite extensive literature review, the manuscript struggles to contextualize its findings. Most importantly, the relationships presented are purely correlational, and are difficult to assume as causal. Protection is postulated as the driving mechanism for carbon protection, but the seasonal inputs and outputs are hardly mentioned. Additional drivers like mineralogy, hydrology, and FT intensity are also not discussed. The influence of these factors has already been described in another manuscript by the same authors, where soil water content was found to be a critical factor (https://doi.org/10.1016/j.catena.2023.107359). This highly related study should be more carefully introduced and discussed in the present work. Moreover, the broader significance of carbon protection in aggregate pores is not strongly established by the manuscript. For example, the study region is generously introduced in the introduction, but does not return in any of the results, discussion, or conclusions. The manuscript could also be improved by a smaller number of better integrated citations. Grammar and paragraph structure

could be improved and streamlined throughout.

We realized the significant impact of other soil factors, especially moisture content, on organic carbon. In other studies, we attempted to compare the impact of soil structure and other factors on SOC. But in this manuscript, we focus on comparing the contributions of different soil structural parameters to carbon fractions.

In our discussion part, we have linked our findings with our previous work, as well as the features of the QTP to ensure that our findings were not out of casualty. For example, in Line 372-378: In the thawing period, pores of <15 μm inhibited the POC loss. Previous studies proved that these pores reduced SOC decomposition via limiting microbial access and shifting microbial metabolism to less efficient anaerobic respiration (Strong et al., 2004; Keiluweit et al., 2017). On the QTP, the positive impact of soil moisture on SOC protection has been revealed in both aggregate scale and landscape scale (Ma et al., 2022; Wang and Hu, 2023). The thawing process is accompanied by an increase in microbial activity and moisture availability, pores of <15 μm are able to hold water surrounding the soil particles (Kim et al., 2021).

To avoid grammatic mistakes and bad organized components, we have thoroughly modified our manuscript. The revised manuscript has been uploaded.

3. I would be interested to see a closer look at the data, with increased focus on the seasonal cycle, causality, and other driving factors. I think the value of the annual time series was not fully explored, and suggest that the analysis could look more carefully at the changes in each layer of each ecosystem over time, rather than aggregating all the soil layers and both ecosystems into the same statistical analysis. For the interesting data and contribution to understanding challenging soil processes, I recommend this manuscript to be reconsidered with revisions to

the analysis, discussion, and contextualization of the findings.

We highly appreciate your insightful suggestions. Analyzing in each layers/ecosystems can indeed yield some interesting results, but in this study, as we focused on the relationship between pores and SOC, more analysis could lead to inconsistent results, which was not conducive to revealing the relationship. In the future research, we would include more indicators to comprehensively reveal the organic carbon protection mechanisms of different soil layers.

Specific Comments

1. The author's previous work in the region should be more thoroughly described and integrated into the manuscript. Discussion of mineralogy, soil water content, and inter-aggregate porosity would all aid in the interpretation of your novel findings here.

Thanks very much for the comments. The related findings have been described and analyzed in the Introduction and Discussion sections. For example, in Line 85-89 (in Introduction): Our previous studies have showed that, alpine meadow soil aggregates of the QTP had dense pore networks with many elongated pores in them due to frequent FT cycles (Zhao et al., 2020). For typical ecosystems on the QTP, the aggregate protection of SOC was promoted by pores of <15 μm by limiting microbial access and the process was most closely associated with soil moisture content (Wang and Hu, 2023).

2. The introduction and conclusion could be strengthened by removing extraneous detail, while focusing more on the implications of the work. Climate change and the QTP is a very

interesting topic, and the reader would be interested in the implications of your work to understanding the future of the region.

Many thanks for the insightful comment. We have added some background and implications in our manuscript. For example, in Line 89-91: “Aggregate stability has been proved to impact SOC protection on the QTP and thawing-induced SOC loss of aggregates will translate into carbon emissions from the meadow to the atmosphere and exacerbate global warming (Ozlu and Arriga, 2021). Also, in Line 395-397: Future research needs to further quantify the impact of soil structure on organic carbon, which will enable us to apply the mechanisms we have discovered to landscape scales to improve existing global carbon cycle predictions.

3. The data on vertical structure (eg Table 1) has potential to be interesting, but is largely unsupported by the manuscript. I suggest it should either be presented with supporting discussion, or trimmed from the manuscript.

Thanks very much for the valuable comment. We have added the related discussion in Line 361-366: Freezing also resulted in a more uniform distribution of SOC across different soil layers. This finding corresponds to Zhao and Hu (2023), which proposed the buffered difference in microbial biomass between soil horizons in the frozen period. The phenomenon may be attributed to differences in external disturbances and SOC turnover rates from topsoil to deep soil (Wang et al., 2022). These indicated the positive effect of freezing on vertical nutrient distribution, which lacks investigations so far.

4. Table 2 and Figure 7 present some interesting correlations, but I would be interested to see a scatter plot (perhaps color-coded by ecosystem) for some of the key relationships. I’m worried

that the seasonal differences reflect different ecosystem behaviors, rather than mechanistic causality.

Many thanks for your valuable suggestions. We have added the scatter plots of some crucial correlations as is shown in Fig. 7 and Fig. 8 and checked whether they reflected ecosystem behaviors. All data were presented as the mean value of each ecosystem in each FT period to avoid error caused by extreme values.

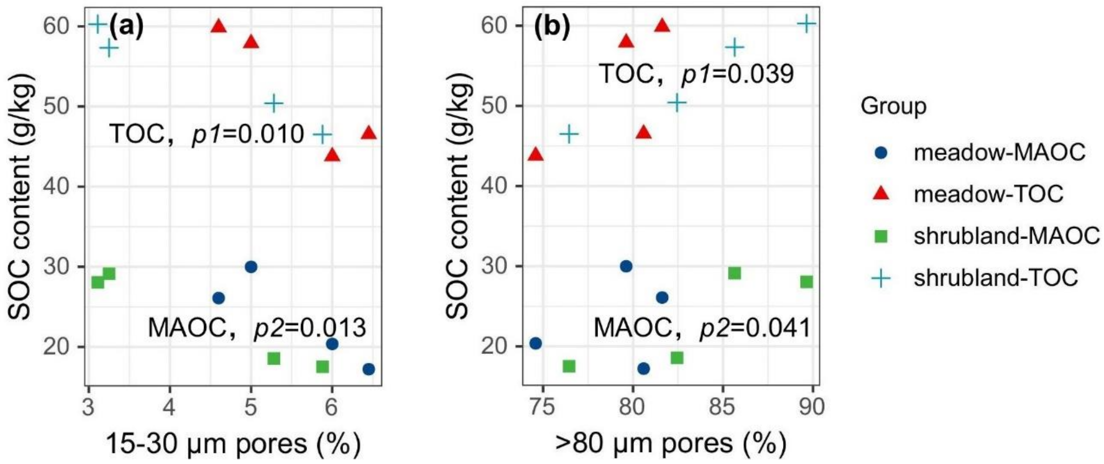

Fig. 7. Scatter plots of relationships between (a) SOC content and 15-30 μm pores and (b) SOC content and > 80 μm pores in the freezing process.

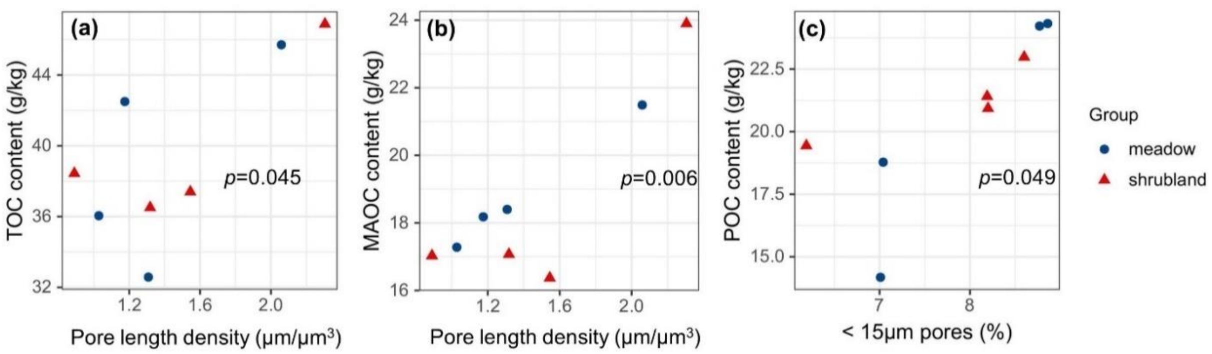

Fig. 8. Scatter plots of relationships between (a) TOC content and pore length density, (b) MAOC content and pore length density and (c) POC content and < 15 μm pores in the thawing process.

5. The results throughout the paper are presented without much discussion of the physical

mechanisms. I think the results in changing pore structure would be much more compelling with thoughtful discussion of the physical mechanisms. The same goes for the mechanisms of carbon protection, considering the sources and sinks of carbon.

We highly appreciate your insightful comments. We have added the discussion of the physical mechanisms, which can be seen in Line 368-390: In the freezing period, pores of 15-30 μm had negative impact on SOC protection, this was consistent with our previous results (Wang and Hu, 2023). Pores of 15–30 μm are probably suitable habitat for soil microbes and support their activity, where greater SOC decomposition takes place (Kravchenko & Guber, 2017; Liang et al., 2019). Pores of >80 μm favoured SOC protection of aggregates. As the period was featured by SOC accumulation (especially residue entry), Pores of > 80 μm serve as primary sites for residue entry and are promoted by microbial materials and SOC, which enhance soil aggregation and thus drive much SOC to be protected (Ananyeva et al., 2013; Dal Ferro et al., 2014; Zhang et al., 2023). Freezing promoted the formation of these pores which were conducive to organic matter entry into aggregates. In the thawing period, pores of <15 μm inhibited the POC loss. Previous studies proved that these pores reduced SOC decomposition via limiting microbial access and shifting microbial metabolism to less efficient anaerobic respiration (Strong et al., 2004; Keiluweit et al., 2017). On the QTP, the positive impact of soil moisture on SOC protection has been revealed in both aggregate scale and landscape scale (Ma et al., 2022; Wang and Hu, 2023). The thawing process is accompanied by an increase in microbial activity and moisture availability, pores of <15 μm are able to hold water surrounding the soil particles (Kim et al., 2021). Therefore, POC associated with these pores was less vulnerable to microbial processing and desorption due to equilibration with the more frequently exchanged soil solution (Schluter et al., 2022). The protection promotes the consequent transport of POC towards mineral sorption and thus contributes to the long-term

SOC storage (Vedere et al., 2020). Overall, the FT-induced pore structure posed a positive impact on SOC protection in that: pores of > 80 μm promoted by freezing serve as primary sites for organic matter entry, while pores of <15 μm promoted by thawing inhibited POC decomposition through holding moisture.

6. Better paragraph structure and organization will improve the overall clarity and readability tremendously. I would be happy to provide more detailed comments on a revised manuscript.

Thanks very much for your insightful opinions. We have improved the organization especially for the Introduction and Discussion sections in the revised manuscript, which will present a better link between our findings and backgrounds of the study