Dear editor

Many thanks for raising the problems and suggestions in our manuscript (ID: egusphere-2024-1833). 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

Thank you to the authors for their revisions, which improved the clarity and quality of the manuscript. The presentation of the methods is quite clear for the most part. The presentation of the data has also been appreciably streamlined. The introduction and especially discussion have both been strengthened in organization and clarity. There are still some areas where I think would be beneficial to clarify, mostly in the discussion section.

Many thanks for acknowledging our revisions and offering valuable suggestions.

Specific topics:

1. The analysis consistently differentiates between meadow and shrubland, or example figures 5,6 and 7. However, the differences between meadow and shrubland are not discussed. Why not either group the data, or discuss the differences? More subtly, looking at meadows vs shrublands, this highlights the difficult of interpreting differences - random heterogeneity, or a systematic signal?

Thanks for raising the valuable question. Soils of the meadow and shrubland ecosystems both belongs to Gelic Cambisols according to the FAO UNESCO system (IUSS Working Group WRB, 2022). These two ecosystems are representative of typical alpine ecosystems on the QTP. These two ecosystems also both have *Kobresia* vegetation and mattic epipedon. The objectives of the study were to quantify changes in pore structure and SOC fraction contents of aggregates in typical alpine ecosystems during the seasonal FT process, and to find the pattern of the effects of FT on pore structure and SOC fraction contents of aggregates as well as their relationships. So, we did not focus on the differences between meadow and shrubland. In the future, we will focus on the differences between these two ecosystems.

2. On a related topic to meadows/shrubs, I am wondering about the difference between pore processes in different size aggregates. Is there any reason to expect different processes in differently sized aggregates, or is this just random heterogeneity? If the processes should be the same between different sized aggregates, perhaps it is fraught to interpret differences between aggregate sizes (eg discussion starting at line 346).

Thanks very much for your valuable question. Soil aggregation processes can be explained by the aggregate hierarchy theory (Tisdall and Oades 1982). Briefly, primary particles and silt-sized aggregates are first bound together into microaggregates by persistent binding agents (e.g. humus, disordered aluminosilicates). Then, several small-sized aggregates are bound together into lager ones by temporary and transient organic binding agents (e.g. fungal hyphae, roots) (Six et al., 2004). Therefore, there are differences in the internal adhesion of aggregates of different sizes. Previous studies have proved that cementing agents (e.g. organic matter and metallic oxide) could affect intra-aggregate pore structure due to its influence on the morphological characteristics, permutation, and the combination of particles (Schweizer et al., 2019; Peng et al., 2022).

Soil aggregates of >0.25-2 mm and>2 mm are both crucial units for SOC protection. In alpine ecosystems, soil pores were formed and developed by complex interactions among root penetration (Hu et al., 2020), FT processes (Zhao et al., 2020), and microbial/animal activities, etc. Considering the differences in stability and internal binding between the two types of aggregates, investigating their pore network can help better evaluate their carbon protection ability.

However, it is pitiful that previous studies have not investigated the pore formation processes of different aggregates. We hope to conduct further investigations in the future.

References:

Hu, X., Li, X., Li, Z., Gao, Z., Wu, X., Wang, P., Lyu, Y., Liu, L.: Linking 3-D soil macropores and root architecture to near saturated hydraulic conductivity of typical meadow soil types in the Qinghai Lake Watershed, northeastern Qinghai-Tibet Plateau. Catena, 185, 104287. https://doi.org/10.1016/j.catena.2019.104287, 2020.

Peng, J., Wu, X., Ni, S., Wang, J., Song, Y., Cai, C.: Investigating intra-aggregate microstructure characteristics and influencing factors of six soil types along a climatic gradient. Catena 210, 105867. https://doi.org/10.1016/j.catena.2021.105867, 2022.

Schweizer, S.A., Bucka, F.B., Graf-Rosenfellner, M., Kogel-Knabner, I.: Soil microaggregate size composition and organic matter distribution as affected by clay content. Geoderma, 355, 113901. https://doi.org/10.1016/j.geoderma.2019.113901, 2019.

Six, J., Bossuyt, H., Degryze, S., Denef, K.: A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res., 79, 7-31. https://doi.org/10.1016/j.still.2004.03.008, 2004.

Tisdall, J.M., Oades, J.M.: Organic matter and water-stable aggregates in soils. J. Soil. Sci., 33, 14-163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x, 1982.

Zhao, Y., Hu, X., Li, X.: Analysis of the intra-aggregate pore structures in three soil types using X-ray computed tomography. Catena, 193, 104622. https://doi.org/10.1016/j.catena.2020.104622, 2020.

3. The discussion of vertical Coefficient of Variation is interesting, but possibly also a good example of correlation vs causation. The manuscript shows very nicely that there are seasonal changes in vertical TOC distribution, but I am skeptical that this is driven by FT processes altering pore structure. It seems more likely that vertical carbon structure and pore structure are both being driven by seasonal changes in hydrology, phenology, and temperature.

We highly appreciate your insightful comment. We agreed with your perspective that the vertical SOC distribution was driven by multiple factors including freeze-thaw, phenology and hydrology, etc., rather than solely FT processes. To avoid ambiguity, we have modified the related discussion into (Line 357-363): The freezing process was also accompanied by a more uniform distribution of SOC across different soil layers. This finding corresponds to Zhao and Hu (2023), which proposed that freezing buffered difference in microbial biomass between soil horizon. Apart from seasonal dynamics in phenology and hydrology, differences in external disturbances and SOC turnover rates from topsoil to deep soil also contributed to this phenomenon (Sun et al., 2020; Wang et al., 2022). Therefore, freezing might pose indirect and positive impact on vertical nutrient distribution, which lacks investigations so far.

4. I appreciate the mention of mineralogy and vegetation as potentially confounding factors, and I think that this discussion can go further. Several parts of the discussion make claims that appear more causal than is warranted, such as "Freezing also resulted in a more uniform distribution of SOC across different soil layers" on line 326-327. - Three is certainly a seasonal cycle, but it feels difficult to claim that freezing is the causal driver. Several instances like this can be improved by emphasizing correlation, not causation.

Many thanks for raising the insightful question. The discussion of mineralogy and vegetation has been added as is shown in Line 397-399: For example, the presence of iron-rich substances can hamper microbial degradation of organic compounds, and the Fe-OC accounted for approximately 20% of the total carbon pool on the QTP (Mu et al., 2016). This mechanism can be closely associated with soil moisture and enzyme activities, both of which are altered by FT processes (Li et al., 2023; Hu et al, 2024). Also, in Line 380-382: Therefore, POC associated with these pores was less vulnerable to microbial processing and desorption as thawing enhanced exchanged soil solution and consequent equilibration (Schluter et al., 2022). We will focus more on soil minerology and vegetation on our following studies.

We highly agreed with you in that “it feels difficult to claim that freezing is the causal driver” and we have revised the expressions to emphasize correlations rather than causation throughout the manuscript. For example (Line 357-358), the freezing process was also accompanied by a more uniform distribution of SOC across different soil layers.

There are a small number of specific corrections, I would be happy to go through and give complete proofreading on the next revision.

Thanks again for your insightful comments. Thanks again for your insightful comments. We have made several revisions concerning the grammatical structure.

Referee #2

The authors have improved the original MS. version, and I thank them for their responses to my initial concerns. However, there are still substantial issues with the revised ms that I will outline below:

Regarding the low sample size and statistical significance, I can appreciate the difficulty and expense of including additional replicates, but my concerns about the reliability and interpretation of statistical significance remain. The authors have not responded to my comment about the data and code availability (they state at the end of the ms that the data are included with the published article) or the multiple comparisons made without statistical adjustment. The number of statistically significant differences discussed in the text are surprising given the apparent lack of differences in the bar plot means. For example, in Fig. 5a, the differences between shrubland >2 mm and shrubland 0.25-2 mm do not appear significant, given the wide SE. Similarly, Fig. 5f meadow >2 and meadow 0.25-2 mm do not appear significantly different. I therefore encourage the authors to double check their calculations.

We highly appreciate your insightful opinions. Due to the scanning accuracy of industrial CT, the sample size was limited. We really hope to expand the sample size to reach a more accurate assessment in future studies if possible.

We have carefully checked our calculations (especially the significant test) and we apologize for any errors existing in previous figures and tables. The revised results can be seen in Fig. 5. The corresponding descriptions were also revised, for example, in Line 252-254: The seasonal FT process did not alter the porosity, pore volume and EqD significantly (Fig. 5a, 5b and 5c). In both ecosystems, significant variations were found in the mean pore volume between >2 mm and 0.25-2 mm aggregates (p<0.05).

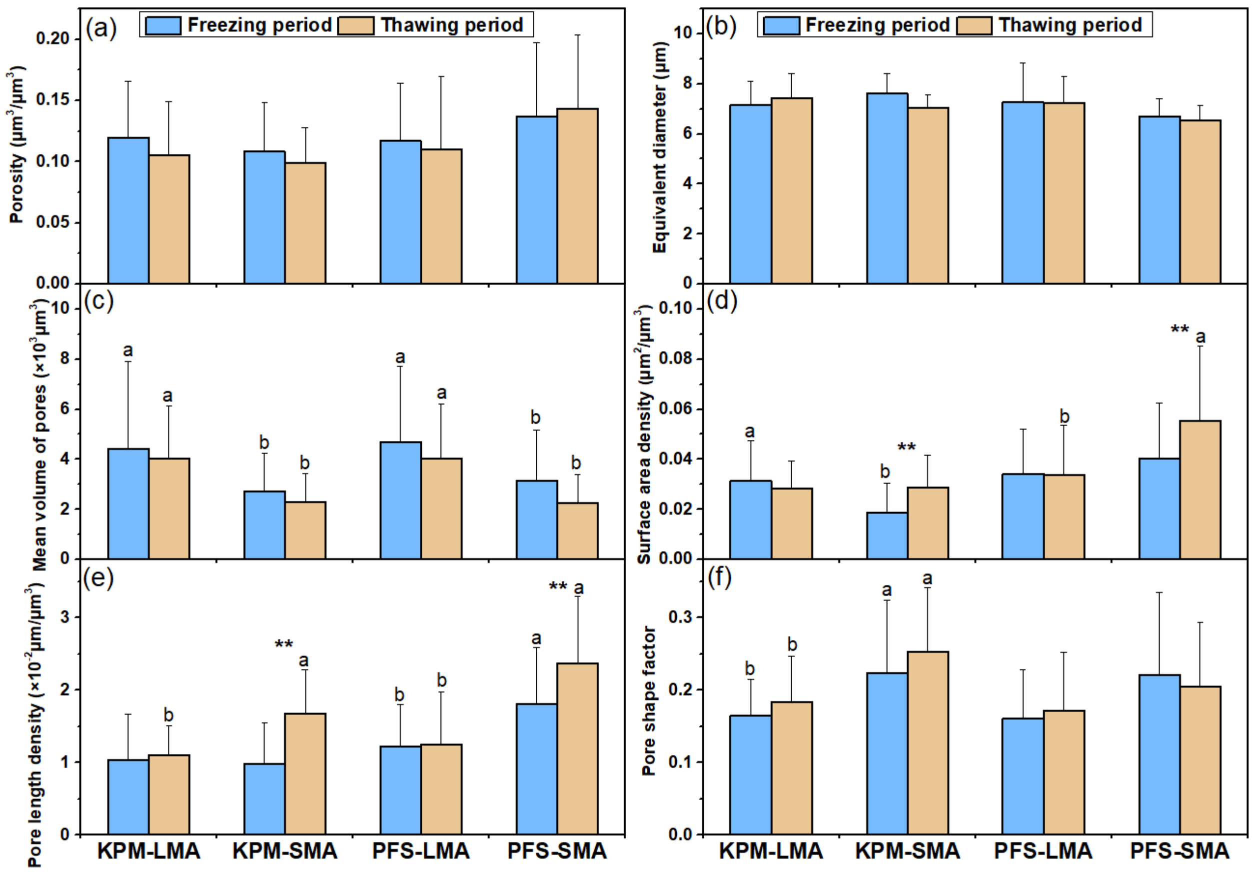

Fig. 5. Pore characteristics of soil aggregates during the seasonal FT process. (a) porosity, (b) pore equivalent diameter, (c) mean volume of pores, (d) pore surface area density, (e) pore length density and (f) pore shape factor. Bars represent the mean ± standard error (n=18). ** represents significant differences between pore characteristics in freezing period and thawing period (p<0.05). Different lowercase letters denote significant differences between pore characteristics of >2 mm aggregates and 0.25-2 mm aggregates (p<0.05).

Note: LMA->2 mm aggregates, SMA-0.25-2 mm aggregates, KPM-the meadow ecosystem, PFS-the shrubland ecosystem.

Specific comments

1. Regarding the below statements in the Abstract (L20-25; L27-30):"The total organic carbon (TOC), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) contents of aggregates were high in the stable frozen period and low in unstable thawing period, demonstrating that freezing process enhanced SOC accumulation while early stage of thawing led to SOC loss. In the freezing period, pore structure inhibited SOC loss by promoting the formation of >80 μm pores. In the thawing period, pores of <15 μm inhibited SOC loss. Our results revealed that changes in pore structure induced by FT processes could positively

contribute to SOC protection of aggregates." I have concerns about the extent to which the data support these assertions. The expanded discussion related to the mechanisms of SOC protection and loss in pores is helpful in the revised ms version, but the relationships between TOC, fraction C, and pore size still remain correlational rather than causational in my view. The wording in the abstract should be changed to reflect the speculative nature of these assertions. In future studies, soil respiration measurements and direct DOC measurements would be a more direct way to capture the losses of soil carbon, although admittedly difficult to capture in situ in freeze-thaw field conditions.

We highly appreciate your valuable comment. We have revised our expressions to emphasize correlations rather than causations. For example, in Line 23-25: demonstrating that freezing process were positively associated with SOC accumulation while early stage of thawing witnessed SOC loss. Also, in Line 26-30: In the freezing period, the SOC accumulation might be enhanced by the formation of >80 μm pores. In the thawing period, pores of <15μm was positively correlated with SOC concentration.

We strongly agree with your views on future research, which has been incorporated into the Discussion part (Line 393-395): Despite the difficulty in in-*situ* monitoring, soil respiration measurements and DOC measurements would be a more direct way to capture the loss pathways of SOC exerted by thawing.

2. L245-246: Since porosity reflects the proportion of soil volume that is made up of pore space (so both number and size of pores), it is probably more correct to state that thawing contributed to an increase in the number of pores of size <15 μm, instead of the porosity of them.

We highly appreciate your insightful suggestion. The related sentence has been modified in Line 239-241: The results showed that freezing process increased the proportions of pores of > 80 μm while thawing contributed to the increase in volume percentage of pores of <15 μm.

3. Fig 4. It would be helpful to again define acronyms in the figure caption.

Thanks very much. The acronyms have been added in the figure caption: Note: UFP-unstable freezing period, SFP-stable frozen period, UTP-unstable thawing period, STP-stable thawed period.

4. Figures 7 and 8 are good additions.

   Many thanks for acknowledging our related revisions.

5. As with the first MS version, there are still many opportunities for improvements in grammatical structure and sentence flow to improve readability, but it is more important that the substantial concerns be addressed first. I remain willing to address specific grammatical issues in subsequent versions.

Thanks very much for your comments improved our work, and the comments are helpful to the improvement of our paper. We have made several revisions concerning the grammatical structure. For example, in Line 399-401: This mechanism can be closely associated with soil moisture and enzyme activities, both of which are altered by FT processes (Li et al., 2023; Hu et al, 2024). Thanks very much for your willingness to address these issues.