

Dear referee

We highly appreciate your valuable comments on our manuscript. We have carefully considered your suggestions and made revisions of our manuscript. The replies are as follows:

#### General comments

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. In total, we scanned 144 aggregates. We have added the data in Line 169. 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 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 The second concern is the soil density fraction method, which is an outdated method from the early 1990s that has proven to be an imprecise method of density separation compared to the more commonly used sodium polytungstate method.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<sup>3</sup>, whereas the commonly agreed upon densities for separating mineral fractions are 1.6 -1.85 g/cm<sup>3</sup>, which cannot be achieved using 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 RDA image has been moved into Supplementary information.

#### 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 which demonstrate a better linkage between our goal and the background. We wish to submit the revised manuscript.

#### 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 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 will be added in the text throughout the revised manuscript. For example, in Line 313-314, “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, 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, which can be seen as follows:

Supplementary Table 1. Basic soil physio-chemical properties

Ecosystem	Soil depth (cm)	Bulk density (g/cm <sup>3</sup> )	Soil water content (%)	pH	Organic C (g/kg)	Total N (g/kg)	Particle size composition (%)		
							clay	silt	sand
KPM (meadow)	0-10	0.77±0.19b	35.76±15.01	6.50±0.35	85.26±29.38a	7.66±2.22a	9.05±2.65	33.60±6.10	57.35±8.73
	10-30	1.00±0.17a	32.00±20.68	6.49±0.19	67.12±20.49ab	6.94±1.37ab	10.65±3.74	35.83±9.05	53.52±12.64
	30-50	1.07±0.05a	24.18±13.04	7.17±0.32	25.35±6.78b	2.66±0.45b	11.84±2.57	34.88±4.98	53.28±7.32
PFS (shrubland)	0-10	0.83±0.23	42.57±4.57a	6.64±0.40	64.42±11.22a	7.00±1.12a	13.95±0.56	47.56±1.25	38.49±1.69
	10-30	0.81±0.15	32.40±8.70ab	6.82±0.22	44.11±6.88ab	4.30±0.90ab	14.59±0.86	46.85±1.00	38.56±1.73
	30-50	0.96±0.15	22.82±0.50a	7.31±0.37	36.44±7.06b	3.38±0.53b	15.05±1.80	47.44±3.80	37.50±5.58

**Note:** KPM-*Kobresia pygmaea* meadow; PFS-*Potentilla fruticosa* shrub. The properties were measured with samples taken in the unstable freezing period. All data is presented with standard error (n=3). Different lowercase letters denote significant difference between soil layers.

8. 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. We hope to get your more detailed suggestions on our revised manuscript.