Dear Editor,

We finished the revision of the manuscript according to the questions and advices of the four reviewers. The following are the details of our responses (in blue color) to questions and advices of every reviewer.
The work of reviewers help improve the quality of the manuscript. We thank the thoughtful advice of the reviewers and hope the revision successfully answered the questions.
Best wishes

Wuchang Zhang
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Reviewer #3 (CC2): The authors presented a detailed and comprehensive dataset of ciliate community distribution across the major temperature zones in the sea, and the ciliate morphospecies were identified in 1,117 samples taken at 175 stations in the Arctic and sub-Arctic Ocean, the North Pacific, the tropical western Pacific, the Indian Ocean, and the Southern Ocean (in global scale). Meanwhile, ciliate abundance and biomass size spectra, as well as species richness and diversity, were related to environmental parameters and depth. Objectives and rationales are clear, robust and well presented. Furthermore, the authors' analyses confirm general trends (e.g., size-diversity and temperature-diversity relationships for aloricate ciliates and tintinnids, a decrease of ciliate abundance and biomass with depth) and present numerous details for each biogeographic zone worth publishing. However, several shortcomings should be reviewed to more fill the scope of their overall goal. In conclusion, I recommend this manuscript for publication in the Ocean Science characterized with high-ranked international journal after revising some specific comments as follow.

Specific comments:
1) Title: pelagic ciliates belonged to Protozoa is well-known in marine plankton realm, thus it's no need to strengthen it in the title. Just delete this term.
Response: We deleted "Protozoa" accordingly **in revised manuscript.**

2) line 48: Common sense error. The "anthropogenic CO2 emissions" should be revised into "anthropogenic CO2 emissions".
Response: We revised into "anthropogenic CO_2 emissions" accordingly **in lines 47–48 in revised manuscript.**
Lines 47–48: Over recent decades, anthropogenic CO_2 emissions have led to increased atmospheric concentrations and greater global radiative forcing (Tagliabue et al. 2023),…

3) line 93: please make sure that whether the cruise conducted in the Indian Ocean in March 2021 aboard the R.V. "Xiangyanghong 10"? I remembered that this cruise

might be conducted by the R.V. “Xiangyanghong 6” in previous manuscript I have reviewed.
Response: After carefully checking, we revised into *R.V.* “Xiangyanghong 6” **in lines 90–92 in revised manuscript.**
Lines 90–92: 4, the Torrid Zone (TZ), which includes the tropical western Pacific in December 2016 and August 2017 aboard the *R.V.* “Kexue”, and the Indian Ocean in March 2021 aboard the *R.V.* “Xiangyanghong 6”.

4) The Methods section lacks detail. I recognized that the method how you calculated the size-fraction of aloricate ciliate, while how the biomass spectra were constructed (size categories?) is unclear. Please state clearly relate to the calculation of the biomass spectra.
Response: We added the calculation of size spectra biomass and revised this sentence accordingly **in lines 122–123 in revised manuscript.**
Lines 122–123: Concerning size spectra biomass, ciliate biomass were calculated based their specific organism volume and conversion equation, then categorized into each size spectrum as in Wang et al. (2024b).

5) line 122: Convert pg C to μg C.
Response: We accepted suggestions and revised into “0.19×10^{-6} μg C μm^{-3}” **in lines 120–121 in revised manuscript.**
Lines 120–121: Additionally, a conversion factor (0.19×10^{-6} μg C μm^{-3}) was used for calculating aloricate ciliate carbon biomass (Putt and Stoecker 1989).

6) line 153: What do you mean the “sandwich structure” for temperature. I cannot find this phenomenon clearly in Figures S1 and S3. Therefore replace it.
Response: We accepted suggestions and revised into “low–high–low structure” **in lines 152–154 in revised manuscript.**
Lines 152–154: Moreover, temperature displayed a low–high–low structure at inner stations of the South Frigid Zone (SFZ), and Chl *a* peaked at subsurface layers in both the North Temperate Zone (NTZ) and TZ (Figures S1 and S3).

7) Figure 2: you have mentioned the abbreviation of the five temperature zones in figure 1: the North Frigid Zone (NFZ), sub-Arctic Zone (SAZ), North Temperate Zone (NTZ), Torrid Zone (TZ) and South Frigid Zone (SFZ), thus there is no need to write this part again.
Response: We accepted suggestions and revised accordingly **in line 174 in revised manuscript.**
Line 174: Figure 2: Variations in environmental variables and ciliate abundance and biomass at discrete depth in each temperature zone.

8) line 275: I wondered that it not clear what is meant by “monospecific trophic levels, such as microzooplanktonic ciliates”; ciliates represent more than one trophic level (i.e., as phototrophs, bacterivores, herbivores/omnivores, predators, parasites). Please

state it clearly in this part.

Response: At this part, we just focused on one group of microzooplanktonic ciliates, thus the words of "monospecific trophic levels" was unseemliness. Based on our viewpoint, we revised into "specific zooplankton assemblage" **in lines 266–267 in revised manuscript.**

Lines 266–267: Currently, research on specific zooplankton assemblage, such as microzooplanktonic ciliates (Wang et al. 2024a), is rarely studied on a global scale.

9) In the discussion part, the author mentioned that the bottom-up control is the resource limitation as previous pointed. In this study, temperature is environmental factor (=environmental filter) for which exert a primary influence..... I strongly suggest to clearly separate in Discussion the interpretation of environmental filters and trophic mechanisms as explanatory variables for the patterns revealed and to make corresponding corrections in the Abstract.

Response: We accepted suggestions and separated the interpretation of environmental filters and trophic mechanisms in the Abstract **in lines 29–31 and lines 317–328 in revised manuscript.**

Lines 29–31: Moreover, a multivariate biota-environment analysis indicated that temperature exert a primary influence on ciliate community constitution in the global marine ecosystem, and the bottom-up control play a key role in shaping assemblages.

Lines 317–328: Furthermore, the Chl *a* functionally serves as a critical ecological mediator in marine food webs, influencing ecosystem stability through both quantitative (abundance) and qualitative (polyunsaturated fatty acid composition) pathways via the fundamental prey-predator interplay (Šolić et al. 2010; Våge and Thingstad 2015; Holm et al. 2022). Consequently, Chl *a* modulated the energy flow of the entire marine ecosystem (Li et al. 2024). As direct micro-grazers of phytoplankton, both the abundance and species richness of ciliates exhibit a significant positive correlation with Chl *a* (Figure 6 and Figures S8–S10), aligning with the aforementioned viewpoint regarding the ecological role of Chl a. As outlined above, coupled with our results about multivariate analyses revealed strong hydrographic-ciliate relationships (Figure 6), while observed trait plasticity in ciliate communities (Yu et al. 2022) further supports the predominance of bottom-up control mechanisms (resource availability, prey quality) (Lu and Weisse 2022; Wang et al. 2023c, 2024c) over top-down regulation (predation pressure from microcrustaceans) (Power 1992; Calbet et al., 2001; Worm and Myers, 2003) in structuring global microzooplankton communities. This trophic cascade pattern underscores the fundamental role of primary production dynamics in governing ciliate population ecology across marine ecosystems.

10) In section 4.3, a recent meta-analysis contradicts the authors' conclusion because ciliate mortality appears to be unaffected by temperature (Weisse, 2024, Limnol. Oceanogr.), which was inconsistent with your results. How do you cope with this phenomenon? By the way, T determines organism mortality contradicts empirical evidence for ciliates (Weisse 2024)

Response: We studied carefully about the recent meta-analysis that ciliate mortality appears to be unaffected by temperature (Weisse, 2024). Regarding this phenomenon, majority previous studies manifested that temperature emerges as a principal driving factor of plankton composition and dispersal, particularly in high-latitude polar regions, due to its direct impact on physiological processes (e.g., respiration, productivity, reproduction) via thermally dependent metabolic regulation (e.g., Knies et al., 2009; Stuart-Smith et al. 2015; Archibald et al., 2022; Chust et al. 2024). In addition, temperature determine the habitat conditions for pelagic plankton. Therefore, we approved the viewpoint that ciliate mortality affected by temperature. We also revised accordingly **in lines 317–328 in revised manuscript.**

References

Knies, J. Kingsolver, J. and Burch, C.: Hotter is better and broader: Thermal sensitivity of fitness in a population of bacteriophages. Am. Nat. 173, 419–430, doi:10.1086/597224, 2009.

Stuart-Smith, R. Edgar, G. Barrett, N. Kininmonth, S. and Bates, A.: Thermal biases and vulnerability to warming in the world's marine fauna, Nature 528, 88–92, doi:10.1038/nature16144, 2015.

Archibald, K. Dutkiewicz, S. Laufkötter, C. and Moeller, H.: Thermal responses in global marine planktonic food webs are mediated by temperature effects on metabolism, J. Geophys. Res. Oceans 127, e2022JC018932, doi:10.1029/2022JC018932, 2022

Chust, G. Villarino, E. McLean, M. Mieszkowska, N. Benedetti-Cecchi, L. Bulleri, F. Ravaglioli, C. Borja, A. Muxika, I. Fernandes-Salvador, J.… and Lindegren, M.: Cross-basin and cross-taxa patterns of marine community tropicalization and deborealization in warming European seas, Nat. Commun. 15, 2126, doi:10.1038/s41467-024-46526-y, 2024.

11) line 316: the author mentioned that the positive correlation between tintinnid species richness and temperature, while this correlation may be an indirect effect.

Response: Our study revealed that tintinnid species richness and temperature had significant positive correlation through biotic-abiotic analysis. This phenomenon was fit the viewpoint that temperature can promote plankton biodiversity through regulating intrinsic temperature-dependent metabolic processes. Regarding this problem, we agreed with the reviewer's point that correlation may be an indirect effect between pelagic ciliate and temperature (Weisse and Sonntag 2016; Weisse 2024). We also revised this sentence accordingly **in lines 309–312 in revised manuscript.**

Lines 309–312: In this perspective, we conclude that temperature determines organism mortality by affecting their thermal affinity within biogeochemical cycles (Knies et al., 2009; Stuart-Smith et al. 2015; Archibald et al., 2022; Chust et al. 2024) through an indirect effect (Weisse and Sonntag 2016; Weisse 2024).

12) line 349: CO2

Response: We revised into "CO_2" **in lines 342–344 in revised manuscript.**

Lines 342–344: Similarly, Benedetti et al. (2021) projected a median speed of approximately 35 km/decade for the poleward shift of species dispersal under a high CO_2 emission scenario by the end of this century.

13) line 360: add the total number of samples.

Response: We added the total number of samples accordingly **in lines 353–355 in revised manuscript.**

Lines 353–355: Our results provides a comprehensive disparities in microzooplanktonic ciliate trait structure focused on size spectrum, biodiversity, and biotic-abiotic interplay based on 1117 water samples from 175 stations across five temperature zones from the North Pole to the Southern Ocean (Antarctic).

14) At last, I'm curious about a phenomenon that the author spend a lot of description in discussing the relationship between the environmental variables and "bottom-up control", and previous studies recognized that the plankton community was strict restricted by outer environmental resources, which was known as "bottom-up control". However, how do you identify the correlation between the environmental variables and "bottom-up control"?

Response: The bottom-up control refers to an ecological mechanism where lower trophic levels (e.g., nutrients, primary producers) regulate the structure and productivity of higher trophic levels (e.g., zooplankton, fish) in marine ecosystems (Lu and Weisse 2022). In other words, bottom-up control can be regarded as a resource-limitated environment. In the marine ecosystem, environmental variables play a key role in reshuffling sophisticated species composition of microbial food web (Lennartz et al. 2024), such as temperature determines organism mortality through modulating their thermal affinity within biogeochemical cycles; Chl *a* directly sustains the stability dynamics of upper trophic levels through providing food items in predation process. Therefore, we consider that the environmental variables and "bottom-up control" are inseparable factors during biotic-abiotic interplay.

References:

Lu, X. and Weisse, T.: Top-down control of planktonic ciliates by microcrustacean predators is stronger in lakes than in the ocean, Sci. Rep. 12, 10501, doi:10.1038/s41598-022-14301-y, 2022.

Lennartz, S. Keller, D. Oschlies, A. Blasius, B. and Dittmar, T.: Mechanisms underpinning the net removal rates of dissolved organic carbon in the global ocean, Global Biogeochem. Cy. 38, e2023GB007912, doi:10.1029/2023GB007912, 2024.