

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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Dear Wuchang Zhang,

We are pleased to inform you that the open discussion of your following OS manuscript on EGUsphere was closed:

Title: Decoding pelagic ciliate (Protozoa, Ciliophora) community divergences in size spectrum, biodiversity and driving factors spanning global five temperature zones

Author(s): Chaofeng Wang et al.

MS No.: egusphere-2024-3888

MS type: Research article

No more referee comments and community comments will be accepted. Now the public discussion shall be completed as follows:

You - as the contact author - are requested to individually respond to all referee comments (RCs) by posting final author comments (ACs) on behalf of all co-authors no later than 09 May 2025 (final response phase). Please log in using your Copernicus Office user ID 322401 at: <https://editor.copernicus.org/egusphere-2024-3888/final-response>

When posting your author comments (ACs), you can choose between new comments or co-listing of existing ones. Please also consider replying to community comments (CCs) from the scientific community.

After your AC posts, you have to explicitly finalize the final-response form through the button "Finalize". You will then receive a separate email asking you to prepare and submit your revised manuscript for peer-review completion and potential final publication in OS.

Preparation and submission of a revised manuscript for peer-review completion is encouraged only if you can satisfactorily address all comments and if the revised

manuscript meets the high quality standards of OS (https://www.ocean-science.net/peer_review/review_criteria.html). In case of doubt, please ask the handling editor directly whether they would encourage submission of a revised manuscript or not.

Please note also that the submission of a revised manuscript does not ensure publication in OS. The editor will carefully assess your revised manuscript in view of the interactive public discussion and may forward it to the original or new referees for further commenting.

You are invited to monitor the processing of your manuscript via the MS records at: https://editor.copernicus.org/EGUsphere/ms_records/egusphere-2024-3888

Thank you very much in advance for your cooperation. In case any questions arise, please do not hesitate to contact me.

Kind regards,

The editorial support team
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Reviewers' comments:

Reviewer #1 (RC1):

The paper “Decoding Pelagic Ciliate (Protozoa, Ciliophora) Community Divergences in Size Spectrum, Biodiversity, and Driving Factors Spanning Five Global Temperature Zones” (Egusphere-2024-3888) studies pelagic ciliates across five temperature zones. Ciliates play a crucial role in the planktonic food web, and expanding our knowledge—especially through studies like this—is essential for understanding their future in the context of climate change.

The ciliate counting work is impressive. The paper is well-written, and the data analysis is highly appropriate. The discussion is engaging; however, some results should be explored in greater depth. Additionally, the discussion contains overly general ideas from the bibliography.

The main revisions should focus on the figures. In the paper, the figures are too small, and some are difficult to interpret. Some figures in the supplementary materials are more effective than those included in the main text. Please select the most appropriate figures to illustrate the results clearly.

For these reasons, I recommend this paper for publication with minor revisions.

Some mistakes:

1) Line 80: field

Response: We revised this previous wrong word into “field” accordingly in lines 79–80 in revised manuscript.

Lines 79–80: By optimizing field observational data and available methods, this study aims to:...

2) Line 73, 87: ciliates

Response: We revised “ciliate” into “ciliates” accordingly in lines 72–75 and lines 86–87 in revised manuscript.

Lines 72–75: As grazer of pelagic phytoplankton, response of microzooplanktonic ciliates to ocean warming in the bipolar and adjacent seas is substantial (Li et al. 2022; Wang et al. 2022a, 2023a, 2023b, 2024b), yet comparative assessments amid their trait structure (e.g., size spectra, biodiversity and biotic-abiotic interplay) remain unexplored to date.

Lines 86–87: Based on their latitudinal locations, field samplings of microzooplanktonic ciliates were conducted in five temperature zones (Trewartha et al. 1967).

3) Line 108: no space after (Utermöhl 1958)

Response: We revised accordingly in lines 105–106 in revised manuscript.

Lines 105–106: After two rounds of siphon process, a final of 25 mL highly concentrated sample was obtained, and then settled in a Utermöhl counting chamber (Utermöhl 1958).

4) Line 141: we used

Response: We revised accordingly in lines 141–143 in revised manuscript.

Lines 141–143: In the following, based on the slope condition, we used the decreasing rate (Δ_D) or increasing rate (Δ_I) according to ciliate abundance or species richness and environmental variables to quantize their interplay in the global seas.

5) Figure 5 and figure 6: legend for a, b, and c

Response: We added legend for previous Figure 5 and Figure 6, and moved these two figures into present Figure S8 and Figure S9 in revised supplementary material in revised manuscript.

Figure S8 (previous Figure 5): Variations in slopes between ciliate abundance and temperature (a)/salinity (b)/Chl *a* (c) at discrete depth in each temperature zone.

Figure S9 (previous Figure 6): Variations in slopes between tintinnid species richness and temperature (a)/salinity (b)/Chl *a* (c) at discrete depth in each temperature zone.

6) Line 368: indirect

Response: We revised this previous wrong word into “indirect” accordingly **in lines 360–361 in revised manuscript.**

Lines 360–361: Under current foreseeable rapid global warming process, we conjecture that bottom-up control (resource limitation) playing a more primary role through an indirect way in the global marine ecosystem.

7) The ciliate counting work is impressive. The paper is well-written, and the data analysis is highly appropriate. The discussion is engaging; however, some results should be explored in greater depth. Additionally, the discussion contains overly general ideas from the bibliography.

Response: Thank you for your appreciation. We realized that some results indeed should be explored in greater depth, and the discussion contains overly general ideas from the bibliography, thus we revised the whole discussion part to fit the scope of this manuscript accordingly **in revised manuscript.**

8) The main revisions should focus on the figures. In the paper, the figures are too small, and some are difficult to interpret. Some figures in the supplementary materials are more effective than those included in the main text. Please select the most appropriate figures to illustrate the results clearly.

Response: We realized that the figures are too small in the manuscript. After careful consideration, we revised previous “Figure 3” into present “Figure 3 and Figure 4”. In addition, we moved previous “Figures 5–6” into present “Figures S8–S9” (Supplementary material) accordingly **in revised manuscript.**

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Reviewer #2 (CC1): The data in this paper are obtained through the accumulation of several scientific cruises, which is very precious and rare. The differences in abundance, biomass, diversity and size spectrum of pelagic ciliate among the five temperature zones were demonstrated through measured data, in addition to the differences between latitudes, the data also showed differences in the vertical patterns of planktonic ciliate abundance, biomass and size structure in the five temperature zones, which is of great value for understanding the global distribution of pelagic ciliate.

1) The analysis on biotic-abiotic interplay is also very meaningful, but the current analysis results in 3.4 are somewhat confused. The main reason is that it is not necessary to show the abiotic factors controlling the spatial variation of ciliates within each temperature zone, because these results are determined by the range of sampling stations in each temperature zone, and are independent of the comparison between the five temperature zones. Thus, the content of part 3.4 and the corresponding discussion

needs to be adjusted. I suggest that PCA focus on analyzing the relationship between the dominant species in the five temperature zones and various abiotic factors.

Response: In order to delete confusion, we moved previous “Figures 5–6” into present “Figures S8–S9” (Supplementary material) accordingly in revised manuscript. Meanwhile, we deleted several sentences to better exhibiting the biotic–abiotic interplay. Regarding the PCA analysis, we want to find out the role of environmental factors played in ciliate composition (both ciliate abundance and species richness) in each temperature zone. The relationship between the dominant species in the five temperature zones and various abiotic factors might be have minimal correlation due to the range of sampling stations in each temperature zone. Consequently, the strategists we have adopted were compared the internal correlation among each temperature zone.

2) Lines 149-150: "At 200 m depth, temperature and Chl *a* peaked in the TZ and North Frigid Zone (NFZ), respectively, deviating from salinity patterns, which exhibited high values in both the TZ and NFZ" The expression of this sentence is not clear, modify it to make it clearer.

Response: We revised this sentence accordingly in lines 149–150 in revised manuscript.

Lines 149–150: At 200 m depth, temperature peaked in the TZ and Chl *a* peaked in the North Frigid Zone (NFZ), contrasting with salinity patterns, which displayed high values in both the TZ and NFZ (Figure 2 and Figure S1).

3) Lines 142-144: The vertical distribution of chlorophyll *a* in SAZ is not described.

Response: We added the vertical distribution of Chl *a* in SAZ in lines 150–152 in revised manuscript.

Lines 150–152: Vertically, both temperature and Chl *a* declined in the NFZ and Sub-Arctic Zone (SAZ) (surface-peak pattern), while salinity increased from the surface to 200 m layers across all regions (Figures S1–S3).

4) Lines 170-171: "Vertically, the large (> 50µm) and small size-fractions exhibited an inverse distribution characteristic across five temperature zones" The meaning of this sentence is not clear, modify it to make it clearer.

Response: We revised this sentence accordingly in lines 170–172 in revised manuscript.

Lines 170–172: Vertically, the relative abundance of the large size-fraction (>50 µm) exhibited a decreasing trend, whereas the small size-fraction displayed an increasing trend across the five temperature zones (Figures S5).

5) Lines 209-240: The large differences in the relationship between biological and abiotic organisms in different temperature zones may be mainly caused by the difference in the selection of sampling areas, rather than the fundamental differences between temperature zones.

Response: We hold the similar viewpoint that the large differences in the relationship

between biological and abiotic organisms in different temperature zones may be mainly caused by the difference in the selection of sampling areas, rather than the fundamental differences between temperature zones. Therefore, the strategists we have adopted were compared the internal correlation among each temperature zone at specific sampling depth (0, 50, 100, and 200 m). In Figure S10, we just want to find out the linear relation between ciliate and each environmental factor at all sampling depth among each temperature zone.

6) Lines 278-279: “the general trend of steeper slopes at the surface compared to the 200 m layer across all regions suggests a community size shift influencing carbon flux efficiency towards higher trophic levels” It is difficult to understand the relationship between the half sentence before and the half sentence after "suggest", and additional explanation is needed.

Response: In order to make this sentence more clear, we added an additional explanation accordingly in lines 269–272 in revised manuscript.

Lines 269–272: Furthermore, the consistently steeper slopes at the surface compared to the 200 m layer across all regions (Figure 3) suggest: (1) a depth-dependent shift in pelagic ciliate community size structure, and (2) greater accessibility of prey for meso-/macro-zooplankton in surface waters compared to the 200 m layer, thereby influencing carbon flux efficiency to higher trophic levels (Stukel et al., 2024).

7) Lines 291-292: “the steeper slopes observed in the abundance size spectra in the bipolar seas compared to the tropical, temperate, and sub-Arctic seas might reflect a prevailing trend towards miniaturization” also, it is difficult to understand the relationship between the half sentence before and the half sentence after "might reflect", and additional explanation is needed.

Response: At present, we find out the phenomenon that the steeper slopes observed in the abundance size spectra in the bipolar seas compared to the tropical, temperate, and sub-Arctic seas, but to be honest, it’s hard for us to explore the explanation. Thus we deleted this sentence in revised manuscript.

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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 CO₂ emissions” accordingly in lines 47–48 in revised manuscript.**

Lines 47–48: **Over recent decades, anthropogenic CO₂ 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 “ $0.19 \times 10^{-6} \mu\text{g C } \mu\text{m}^{-3}$ ” in lines 120–121 in revised manuscript.**

Lines 120–121: **Additionally, a conversion factor ($0.19 \times 10^{-6} \mu\text{g C } \mu\text{m}^{-3}$) 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 “CO₂” **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 CO₂ 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-limited 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.

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Reviewer #4 (CC3): The manuscript "Decoding pelagic ciliate (Protozoa, Ciliophora) community divergences in size spectrum, biodiversity and driving factors spanning global five temperature zones" by Wang and collaborators uses an impressive dataset on the distribution of pelagic ciliates over different ecological regions, describing important community features such as size and species composition using environmental parameters to contextualize their findings. While the data is fantastic and should be published, the current version of the manuscript still needs further work. I provide some specific comments below:

1) The language should be revised and the text can be streamlined in several parts. A clear example is the title, which is rather long and not really informative.

Response: We accepted suggestions and revised the whole manuscript accordingly. As for title, it conveyed the three main themes of this manuscript, thus we revised into “Decoding pelagic ciliate (Ciliophora) community divergences in size spectrum, biodiversity and driving factors spanning global five temperature zones” in revised manuscript.

2) Considering that you target only one planktonic group, maybe the normalized size spectra approach is not the best to describe the variation in sizes (as also discussed by the authors in the manuscript). Could simple metrics, such as the average size be more informative?

Response: We agreed with your viewpoint that the normalized size spectra approach is not the best to describe the variation in sizes for only one planktonic group. While regarding different temperature zones, there were several variations for pelagic ciliates lived in oceanic habitat. We tried to find out their divergences in size spectra aspect. Actually, we used the average size of each ciliate size-fraction (for instance, we used the 15 µm size-fraction in 10–20 µm size-fraction) in size spectrum analysis in the manuscript. We also revised accordingly in lines 114–115 in revised manuscript.

Lines 114–115: Furthermore, we select the average value (15, 25, 35, 45 μm ,..., etc) of each size-fraction of both loricate ciliate and tintinnid as the counting criterion for ciliate size spectra (Wang et al. 2024b).

3) The authors should also consider other traits than size to describe the communities, such as the presence/absence of lorica and trophic mode could be more meaningful than the normalized size spectra.

Response: Dear reviewer, thank you very much for proposing these valuable suggestions (the presence/absence of lorica and trophic mode) for pelagic ciliate trait study. To be honest, we counted the presence/absence of tintinnid lorica only in recent two years (starting from 2023 in the Arctic Ocean). Thus relative data was not recorded in the Bering Sea, North Pacific, tropic western Pacific and Indian Ocean. Therefore, we can not conduct this trait structure. Concerning trophic mode, pelagic ciliate belonged to the top grazer of the microbial food web. To date, we already start to study its role in the microbial food web, and relative study in tropic western Pacific have been published in Marine Pollution Bulletin. The other one relate to the Arctic Ocean just submitted in the Global Biogeoscience Cycles. In the near future, we will put more emphasis on uncovering trophic mode of pelagic ciliate in marine ecosystem.

4) I have reservations about how the statistical methods were used by the authors. A constrained ordination using the entire data set might be more appropriate than the ordination analysis. In addition, the relationship between the community and environmental variables could be done with a more comprehensive model (e.g. GLM that also includes zone and depth as independent variables).

Response: Dear reviewer, we appreciate for your valuable advice that using the entire data set might be more appropriate than the ordination analysis. To be honest, we hold the similar viewpoint with reviewer 2 that the large differences in the relationship between biological and abiotic organisms in different temperature zones may be mainly caused by the difference in the selection of sampling areas, rather than the fundamental differences between temperature zones. Therefore, the strategies we have adopted were compared the internal correlation among each temperature zone at specific sampling depth (0, 50, 100, and 200 m). In Figure S10, we just want to find out the linear relation between ciliate and each environmental factor at all sampling depth among each temperature zone. Additionally, we are really sorry that we did not conduct a comprehensive GLM model due to our complex data in both latitudinal and vertical directions, thus we have no idea on how we conduct this model.

5) Considering that seasonality is also important to modulate protozoan communities, are all the datasets comparable in this regard?

Response: We acknowledged that seasonality is important to modulate protozoan communities, but this phenomenon was obvious in both temperate and polar seas. Regarding tropic seas in both the Pacific and Indian Ocean, the community structure including vertical distribution pattern, abundance and biomass values, species composition were almost same (e.g., Sohrin et al., 2010; Li et al., 2018; Wang et al.,

2019a, 2020, 2022b). In other words, seasonality might not be a driving factor for pelagic ciliate community in tropic seas.

References:

- Sohrin, R. Imazawa, M. Fukuda, H. and Suzuki, Y. Full-depth profiles of prokaryotes, heterotrophic nanoflagellates, and ciliates along a transect from the equatorial to the subarctic central Pacific Ocean. *Deep-Sea Res. II* 57, 1537–1550. doi:10.1016/j.dsr2.2010.02.020, 2010.
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- Wang, C. Li, H. Zhao, L. Zhao, Y. Dong, Y. Zhang, W. and Xiao, T.: Vertical distribution of planktonic ciliates in the oceanic and slope areas of the western Pacific Ocean, *Deep-Sea Res. II* 167, 70–78, doi:10.1016/j.dsr2.2018.08.002, 2019a.
- Wang, C. Li, H. Xu, Z. Zheng, S. Hao, Q. Dong, Y. Zhao, L. Zhang, W. Zhao, Y. and Xiao, T.: Difference of planktonic ciliate communities of the tropical West Pacific, the Bering Sea and the Arctic Ocean, *Acta Oceanol. Sin.* 39, 9–17, doi:10.1007/s13131-020-1541-0, 2020.
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- Wang, C. Zhao, Y. Du, P. Ma, X. Li, S. Li, H. Zhang, W. and Xiao, T.: Planktonic ciliate community structure and its distribution in the oxygen minimum zones in the Bay of Bengal (eastern Indian Ocean), *J. Sea Res.* 190, 102311, doi:10.1016/j.seares.2022.102311, 2022b.

6) Some of the sampling campaigns occurred over relatively large areas, which could be sampling over systems divided by oceanographic features, such as fronts. Was the intra-zone variability taken into account?

Response: We aware that sampling campaigns occurred over relatively large areas and the marine pelagic ciliate community exhibited some differences by oceanographic features. Actually, the intra-zone variability was taken into account during writing process. In a whole, compared to different temperature zones, we found that the intra-zone variability of ciliate community was negligible.