Dear Editor,

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

Wuchang Zhang
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Public justification (visible to the public if the article is accepted and published):
Dear Dr. Wang and co-authors,

Your revised manuscript has much improved. Both referees are satisfied with your response to their reviews.

I have read your revised manuscript and come to a list of comments below. First, there are three comments by a referee (first three in my listing below) that I think should also find a way into the manuscript.

My impression of the manuscript is that the data and results are great. I think there is more discussion in all of these data. You could go deeper into these and I hope and encourage you to expand the discussion to some extent.

With kind regards
Mario Hoppema

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Reviewers' comments:
1. The third point by referee #4 (CC3):
"3) The authors should also consider other traits than size to describe the communities, such as the presence/absence of lorica and trophy mode could be more meaningfull than the normalized size spectra."
I agree with the referee. Your answer to the comment is agreed, but you should also mention these points in the manuscript.
Response: We added the information about the presence/absence of lorica and trophy mode accordingly **in lines 113–114 and lines 267–268 in revised manuscript.**
Lines 113–114: Moreover, we did not distinguish the presence/absence of tintinnid lorica during the sample counting process.
Lines 267–268: Additionally, more emphasis should be put on uncovering trophy mode of pelagic ciliate in marine ecosystem.

2. The fourth comment by referee #4 (CC3):

"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."

Again, your answer is agreed, but I would like to find it in the manuscript as well.

Response: We added the information accordingly **in lines 141–144 in revised manuscript.**

Lines 141–144: Moreover, in order to reduce deviation in the relationship between biological and abiotic 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, the internal correlation among each temperature zone at specific sampling depth (0, 50, 100, and 200 m) were compared in the following text.

3. The fifth comment by referee #4 (CC3):

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

Again, please add this info to the manuscript.

Response: We added the relevant information about seasonality and references accordingly **in lines 135–138 in revised manuscript.**

Lines 135–138: Finally, although 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).

4. Title: I think a slight modification (globally vs. global) would make it better: Decoding pelagic ciliate (Ciliophora) community divergences in size spectrum, biodiversity and driving factors globally spanning five temperature zones

Response: We accepted suggestions and revised the title accordingly **in lines 1–3 in revised manuscript.**

Lines 1–3: Decoding pelagic ciliate (Ciliophora) community divergences in size spectrum, biodiversity and driving factors globally spanning five temperature zones.

5. L26-27 “Moreover, ciliate size spectra exhibited a decrease trend from small to large size spectra, with steeper slopes observed in bipolar zones (NFZ and SFZ) compared to the other temperature zones.” This sentence is not clear. A decreasing trend from small to large sizes spectra is confusing and an arbitrary reader does not understand this. And which slope is meant here, which is getting steeper? This needs more explanation.

Response: In order to make this sentence more clearly, we revised “slope” (data of the size spectrum at specific temperature zone) into “slope line” (comparable tendency for evaluating the decreasing trend from small to large size spectrum) accordingly **in lines 26–28 in revised manuscript.**

Lines 26–28: Moreover, although abundance of ciliate size spectra exhibited a decrease trend from small to large size spectra globally, the steeper slope lines observed in both polar zones (NFZ and SFZ) compared to the other temperature zones.

6. L27 “in bipolar zones”, change to: in both polar zones
Response: We revised the previous “in bipolar zones” into “in both polar zones” accordingly **in lines 26–28 in revised manuscript.**
Lines 26–28: Moreover, although abundance of ciliate size spectra exhibited a decrease trend from small to large size spectra globally, the steeper slope lines observed in both polar zones (NFZ and SFZ) compared to the other temperature zones.

7. L29 “while bipolar seas”, change to: while the polar seas
Response: We revised the previous “while bipolar seas” into “while the polar seas” accordingly **in lines 28–29 in revised manuscript.**
Lines 28–29: Latitudinally, ciliate abundance and tintinnid biodiversity exhibited an anti-phase relationship, where the TZ hosted peak biodiversity while the polar seas showed the highest abundance.

8. L30 “exert a primary influence on ciliate community constitution” But how does it exert influence?
Response: We revised “exert” into “play” accordingly **in lines 29–31 in revised manuscript.**
Lines 29–31: Furthermore, a multivariate biota-environment analysis indicated that temperature play a primary influence on ciliate community constitution in the global marine ecosystem, and the bottom-up control play a key role in shaping assemblages.

9. L32-33 “can be generalised for assessing the potential effects of climate change on pelagic microzooplankton in future marine realm.” Is there any evidence for this in the paper? If not, then this contention should be toned down.
Response: We revised previous “microzooplankton” into “ciliates” to toned down the contention accordingly **in lines 31–33 in revised manuscript.**
Lines 31–33: In conclusion, these results underscore the unprecedented divergences in ciliate trait structure among five temperature zones and can be generalised for assessing the potential effects of climate change on pelagic ciliates in future marine realm.

10. L38-41 A verb is missing in this sentence. Therefore, insert exists: Albeit a myriad of prevailing research exists relevant to …
Response: We accepted suggestions and revised accordingly **in lines 38–41 in revised manuscript.**
Lines 38–41: Albeit a myriad of prevailing research exists relevant to plankton biogeography and its interplay with environmental drivers highlighting its importance

in disentangling marine ecosystems and biogeochemical cycles (e.g., Wang et al. 2020; Darnis et al. 2022; Segaran et al. 2023; Tagliabue et al. 2023).

11. L48 Tagliabue et al. 2023 is not the correct reference at this place.
Response: We changed "Tagliabue et al. 2023" into "Singh et al. 2021" 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 (Singh et al. 2021), triggering diverse ecological feedbacks worldwide….

12. L52 polar instead of bipolar
Response: We revised "bipolar" into "polar" accordingly **in lines 51–53 in revised manuscript.**
Lines 51–53: In this sense, extensive existing studies put emphasis on biotic community response to climate change in the polar and adjacent seas owing to their higher susceptibility compared to tropical, subtropical, and temperate seas.

13. L68 "holopelagic species that project the relevant adaptative strategies" It is not clear what project of strategies implied? Please explain and use different wording.
Response: We revised this sentence accordingly **in lines 67–68 in revised manuscript.**
Lines 67–68: Recent escalation in global warming have imposed a cascade of impacts on aquatic ecosystems, presenting a formidable challenge to inherent holopelagic species that modify their relevant adaptative strategies.

14. L73 polar instead of bipolar
Response: We revised "bipolar" into "polar" accordingly **in lines 72–73 in revised manuscript.**
Lines 72–73: As grazer of pelagic phytoplankton, response of microzooplanktonic ciliates to ocean warming in the polar and adjacent seas is substantial.

15. L78 delete sophisticated, as it does not fit here
Response: We accepted suggestions and deleted this word accordingly **in lines 76–78 in revised manuscript.**
Lines 76–78: Consequently, elucidating microzooplanktonic ciliate size spectra, species diversity and biotic-abiotic interplay at a global-scale is critical for projecting future marine ecosystem dynamics, particularly given their unresolved role in plankton response to climate changes.

16. L82 delete process, as it is not necessary here
Response: We deleted this word accordingly **in lines 81–83 in revised manuscript.**
Lines 81–83: Given the current foreseeable rapid climate change, this study will offer a benchmark for facilitating the phenological and bioclimatic progression of microzooplankton shifts in future global marine ecosystem realm.

17. L82 benchmark instead of valuable norm

Response: We accepted suggestions and revised into "benchmark" **in lines 81–83 in revised manuscript.**

Lines 81–83: Given the current foreseeable rapid climate change, this study will offer a benchmark for facilitating the phenological and bioclimatic progression of microzooplankton shifts in future global marine ecosystem realm.

18. L95 Please give the precision of the measurements of temperature, salinity and chlorophyll a in vivo fluorescence.

Response: Dear reviewer, to be honest, we did not know the precision of the measurements of temperature, salinity and chlorophyll *a* in vivo fluorescence by the CTD (SeaBird SBE 911). After each cruise, all above-mentioned environmental data were recorded by the CTD during its combined sampling process.

19. Figure 1 caption: polar instead of bipolar. Add the definition of AO used in the figure

Response: We accepted suggestions and revised "bipolar" into "polar" **in lines 101–102 in revised manuscript.** In addition, we revised "AO" into "Arctic Ocean" **in revised Figure 1 in revised manuscript.**

Lines 101–102: Figure 1: Survey stations and transects (Tr.) in the tropical, temperate and polar seas. NFZ, North Frigid Zone; SAZ, Sub-Arctic Zone; NTZ, North Temperate Zone; TZ, Torrid Zone; SFZ, South Frigid Zone.

20. L120 Please use present tense here: C is the …, V is the…

Response: We accepted suggestions and revised accordingly **in line 121 in revised manuscript.**

Line 121: Where C (10^{-6} μg C) is the carbon biomass of individual tintinnid, V_i (μm^3) is the lorica volume.

21. L123-124 Something is wrong in this sentence. I think the word "test" should be deleted. Please check.

Response: We deleted the word "test" **in revised manuscript.**

22. L155-157 This is a lot of info which is not well-arranged. This info would be better presented in a table. Info later on in the text could also be included in such table.

Response: We accepted suggestions and added a table named "Table S1" accordingly **in revised Supplementary material.**

23. Figure 2 and caption (similar Figures 3 and 4): Please write in the caption which depths are shown, for example, … in panels from top to bottom 2m, etc.

Response: We accepted suggestions and revised accordingly **in lines 163–164, lines 203–204, and lines 206–207 in revised manuscript.**

Lines 163–164: Figure 2: Variations in environmental variables and ciliate abundance and biomass at discrete depth (2, 50, 100 and 200 m) in each temperature zone.
Lines 203–204: Figure 3: Variations in body-size spectra of ciliate normalized abundance at discrete depth (2, 50, 100 and 200 m) in each temperature zone.
Lines 206–207: Figure 4: Variations in body–size spectra of ciliate normalized biomass at discrete depth (2, 50, 100 and 200 m) in each temperature zone.

24. L177-179 "Generally, the slopes of the normalized abundance and biomass size spectra varied from -2.13 to -0.87 (average -1.60±0.33), and from -0.99 to -0.08 (average -0.53±0.25), respectively, with the former was much steeper than the latter (Figure 3)." This sentence is really hard to understand. Please split the sentence in two or three and explain exactly what you mean.
Response: We revised this sentence accordingly **in lines 186–188, lines 188–190, and lines 196–198 in revised manuscript.**
Lines 186–188: Generally, the slopes of the normalized abundance size spectra varied from -2.13 to -0.87 (average -1.60±0.33), and relevant biomass values varied from -0.99 to -0.08 (average -0.53±0.25), with the former slope line was much steeper than the latter.
Lines 188–190: Therein, ciliate abundance decreased from small (15 μm) to large size spectra (> 100 μm), with the slope line of the normalized abundance size spectra in both the NFZ (-2.13 to -1.93, average -2.01±0.09) and SFZ (-2.01 to -1.63, average -1.80±0.17) being steeper than in the other three regions at each depth.
Lines 196–198: Moreover, the slope lines of the normalized biomass size spectra in the SFZ (-0.99 to -0.77, average -0.86±0.10) were steeper than that in the SAZ (-0.74 to -0.43, average -0.62±0.13), NTZ (-0.63 to -0.44, average -0.53±0.09), TZ (-0.74 to -0.25, average -0.47±0.22) and NFZ (-0.37 to -0.08, average -0.21±0.12) (Figure 4).

25. L197-198 Please explain how the Margalef and Shannon indices are defined and what they are used for, as some readers are probably not familiar with that.
Response: We added the usages of both Margalef and Shannon indices accordingly **in lines 209–211 in revised manuscript.**
Lines 209–211: Tintinnid assemblages exhibited significant spatial heterogeneity in both species richness and diversity metrics (Margalef index–d_{Ma} and Shannon index–H_2' are quantitative measures of species richness in ecological communities) across five temperature zones.

26. L223 polar instead of bipolar
Response: We revised "bipolar" into "polar" accordingly **in lines 236–237 in revised manuscript.**
Lines 236–237: In addition, only the polar seas exhibited an increasing trend ($\Delta_1 \geq 0.01$) in species richness–Chl *a* correlation at each sampling layer (Figure S9).

27. L252-253 The sentence "Additionally, … and SFZ." Does not appear to fit here and should be deleted.

Response: We accepted suggestions and deleted the previous sentence **in revised manuscript.**

28. L254-255 “However, the current dataset remains geographically constrained, particularly lacking representation from Atlantic Ocean ecosystems …” This is an important contention which could be further discussed. Is there any work on ciliates done in the Atlantic which can be compared with the present study?
Response: Dear reviewer, to our knowledge, only Li et al. (2023) studied the tintinnid assemblage in the Atlantic Ocean as listed in the following text. However, this work on ciliates done in the Atlantic can not be compared with the present study due to the differences in sampling process. During the sampling process, Li et al. (2023) just got tintinnid samples from DCM to surface layers combined with 5-20 L of water gently filtered through a 10 mm mesh net. Unlike to our sampling process in the manuscript, this sampling method will cause greatly damage to aloricate ciliates (dominant group among pelagic ciliate globally). Therefore, we wrote that “However, the current dataset remains geographically constrained, particularly lacking representation from Atlantic Ocean ecosystems where ciliate communities may exhibit distinct adaptive strategies” **in lines 266–267 in revised manuscript.**.

Li, H., Tarran, G. A., Dall’Olmo, G., Rees, A. P., Denis, M., Wang, C., Gregori, G., Dong, Y., Zhao, Y., Zhang, W., and Xiao, T.: Organization of planktonic Tintinnina assemblages in the Atlantic Ocean. Front. Mar. Sci. 10, 1082495. doi:10.3389/fmars.2023.1082495, 2023

29. L264 change to: … the majority of integrative analyses …
Response: We accepted suggestions and revised accordingly **in lines 276–277 in revised manuscript.**
Lines 276–277: the majority of integrative analyses have primarily focused on biomass density within the size spectrum rather than on the abundance distribution across different trophic levels.

30. L268 polar instead of bipolar
Response: We revised “bipolar” into “polar” accordingly **in lines 279–280 in revised manuscript.**
Lines 279–280: our study revealed that the slopes of abundance size spectra in both the NFZ and SFZ were steeper in polar seas than other three regions latitudinally.

31. L288 polar instead of bipolar
Response: We revised “bipolar” into “polar” accordingly **in lines 299–300 in revised manuscript.**
Lines 299–300: Consistent with both observational and modeling studies, tintinnid biodiversity was highest in the tropical and subtropical seas, and was lowest in the polar seas.

32. L289-290 These are really many references for this contention. Maybe some of these can be discarded.
Response: We deleted five references **in lines 299–302 in revised manuscript.**
Lines 299–302: Consistent with both observational and modeling studies, tintinnid biodiversity was highest in the tropical and subtropical seas, and was lowest in the polar seas (Figure 5) (e.g., Sherr et al. 1997; Dolan et al. 2014, 2016; Righetti et al. 2019; Benedetti et al. 2021; Wang et al. 2020, 2024a; Li et al. 2016, 2018, 2022).

33. L292-293 change to: … more diversified phytoplankton is probably responsible for …
Response: We accepted suggestions and revised this sentence accordingly **in lines 304–306 in revised manuscript.**
Lines 304–306: more diversified phytoplankton in tropical zone (Tian et al., 2024) is probably responsible for subsequent higher tintinnid biodiversity compared to polar zones through endosymbiosis mechanism (Margulis and Sagan 2002; Clark et al. 2023).

34. L293 polar instead of bipolar
Response: We revised "bipolar" into "polar" accordingly **in lines 304–306 in revised manuscript.**
Lines 304–306: more diversified phytoplankton in tropical zone (Tian et al., 2024) is probably responsible for subsequent higher tintinnid biodiversity compared to polar zones through endosymbiosis mechanism (Margulis and Sagan 2002; Clark et al. 2023).

35. L292 "more diversified phytoplankton" Is that only existent in the tropical regions and not in the polar regions? That would need a reference
Response: We meant that more species richness occurred in the tropical regions compared to polar seas. In order to make this sentence more clear, we added several references accordingly **in lines 303–306 in revised manuscript.**
Lines 303–306: After a long-term genetic DNA exchange and evolution process driven by closely prey-predation interaction (Chen et al. 2012), more diversified phytoplankton in tropical zone (Tian et al., 2024) is probably responsible for subsequent higher tintinnid biodiversity compared to polar zones through endosymbiosis mechanism (Margulis and Sagan 2002; Clark et al. 2023).

36. L296-299 "Generally, large-scale hydrographic features, particularly oceanic gyres and distinct water masses, create biogeographic discontinuities that disrupt ecological connectivity despite physical ocean connectivity (Yang et al. 2020). These mesoscale structures establish unique ecoregions with characteristic environmental sensitivities (Longhurst 2007), …" This is important information. However, the interesting question would be if this also plays a role in the explanation of the structures in your observation? Please explain this in somewhat more detail.
Response: Our study indicated that tintinnid biodiversity was highest in tropical,

subtropical, temperate and polar seas might be due to physical barriers formed by oceanic gyres and distinct water masses. In order to make this sentence more clear, we revised this part accordingly **in lines 308–315 in revised manuscript.**

Lines 308–315: Generally, large-scale hydrographic features, particularly oceanic gyres and distinct water masses, create biogeographic discontinuities that disrupt ecological connectivity despite physical ocean connectivity (Yang et al. 2020). These mesoscale structures establish unique ecoregions with characteristic environmental sensitivities (Longhurst 2007), as evidenced by pronounced tintinnid community differentiation across the North Pacific Gyre, Subarctic Gyre, and Beaufort Gyre systems (Wang et al. 2020). Therein, our results revealed that tintinnid biodiversity was highest in the tropical (West Pacific and Indian Ocean) and temperate (North Pacific) seas, then followed by the Sub-Arctic (Bering Sea) and polar seas (Arctic Ocean and Southern Ocean around Antarctic) (Figure 5) were consistent with Wang et al. (2020), proved that plankton biogeography were deeply affected by oceanic gyres.

37. L310 “by affecting their thermal affinity within biogeochemical cycles” It is not clear to me what is meant here. Please explain and rephrase.

Response: For biota, temperature can promote their activity level through regulating intrinsic temperature-dependent metabolic processes. When the outer temperature were higher or lower than an organism’s tolerance range, then the final ending for the organism was die. We aware that this sentence was not clear, and rephrased accordingly **in lines 321–324 in revised manuscript.**

Lines 321–324: Conventionally, temperature can impact plankton biodiversity through regulating intrinsic temperature-dependent metabolic processes, which further determined that which kind of species can live in such a specific temperature environment (Archibald et al. 2022; Lukić et al. 2022; Weisse 2024).

38. L314-316 “Our study, along with others, indicates that ciliate inhabiting higher salinity environments in both the TZ and NTZ (Figure S8) compared to bipolar regions might be a reflection of their 315 higher osmotic pressure affinity.” This is an interesting conclusion. However, is there any indication that these relatively small salinity difference do play such a role? Are there laboratory experiments available to show that?

Response: Dear editor, targeted this problem, we just found that ciliate lived in a higher salinity environments in both the TZ and NTZ than polar seas (Figure S8), thus we made a speculation for this phenomenon. There were no relative laboratory experiments available to show the above-mentioned phenomenon.

39. L315 polar instead of bipolar

Response: We revised “bipolar” into “polar” accordingly **in lines 330–332 in revised manuscript.**

Lines 330–332: Our study, along with others, indicates that ciliate inhabiting higher salinity environments in both the TZ and NTZ (Figure S8) compared to polar regions might be a reflection of their higher osmotic pressure affinity.

40. L317-319 “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 …” I do not understand what the authors are saying here. All phytoplankton have chlorophyll, so why would chl be the mediator? And what has chl to do with the polyunsaturated fatty acid composition? Please explain.
Response: We revised this sentence accordingly in order to state the ecological function of Chl *a* accordingly **in lines 333–335 in revised manuscript.**
Lines 333–335: Furthermore, the Chl *a* functionally serves as the food resource in marine food webs, influencing ecosystem stability through both quantitative (abundance) and qualitative (nutrient composition) pathways via the fundamental prey-predator interplay (Šolić et al. 2010; Våge and Thingstad 2015; Holm et al. 2022).

41. L319-320 “Consequently, Chl a modulated the energy flow of the entire marine ecosystem (Li et al. 2024).” This is trivial, because all energy in the system in the end originates from phytoplankton and its chl.
Response: We deleted this sentence **in revised manuscript.**

42. L326-327 “in structuring global microzooplankton communities.” Your results cannot just be extrapolated to all microzooplankton. You can only contend this for the ciliates in your study.
Response: We accepted suggestions and revised accordingly **in lines 338–342 in revised manuscript.**
Lines 338–342: 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 pelagic ciliate communities.

43. L327-328 “This trophic cascade pattern underscores the fundamental role of primary production dynamics in governing ciliate population ecology across marine ecosystems.” This sounds trivial. Maybe you can be more specific about what you mean here.
Response: We deleted this sentence accordingly **in revised manuscript.**

44. L330 delete industrial-induced
Response: We deleted “industrial-induced” accordingly **in revised manuscript.**

45. L340 delete full sentence as this was already stated in the previous paragraph
Response: We deleted this full sentence (previous L340) **in revised manuscript.**

46. L342 delete Similarly
Response: We deleted “Similarly” **in revised manuscript.**

47. Discussion 4.4 This is an interesting discussion. However, I would expect that the direct results by the authors would play a bigger role in this discussion. I encourage the authors to expand the discussion to some extent to include concrete ciliate results
Response: We added a sentence and revised this part accordingly **in lines 362–365 in revised manuscript.**
Lines 362–365: Moreover, combined with our results that only the NFZ and SAZ exhibited an increasing trend ($\Delta_I \geq 0.03$) in abundance–emperature correlation at surface layers compared with other three zones (Figure S9), we predict that the pelagic surface‐dweller ciliates in both the sub-Arctic and Arctic seas will benefit from the future global warming..

48. L358 polar instead of bipolar
Response: We revised “bipolar” into “polar” accordingly **in lines 373–374 in revised manuscript.**
Lines 373–374: Additionally, tintinnid biodiversity was highest in tropical and subtropical seas and lowest in polar seas.

49. All references: Please place a comma after the author’s initial. For example, the first reference will then read: Amargant-Arumí M., Müller, O., Bodur, Y., Ntinou, I., Vonnahme, T., Assmy, P., Kohlbach, D., Chierici, M., Jones, E., Olsen, L., Tsagaraki, T., Reigstad, M., Bratbak, G., and Gradinger, R.:
Response: We added a comma after the author’s initial throughout all references **in revised manuscript.**