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10th Oct 23

Dear Professor Ji,

Your manuscript titled "Long-term wetland biomonitoring highlights the different impacts of anthropogenically induced factors on regional macroinvertebrate diversity in Dongting Lake, China" has now been seen by 2 reviewers, and we include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. In particular, we require that you:

- * Consider and discuss the direct and indirect influence of land use change to explain multi-linear relations.
- * Tone down your claims to reflect the specificity of your analysis and the insights gained from it.

Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Please don't hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and the completed checklist:

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Please do not hesitate to contact us if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Huai Chen, PhD
Editorial Board Member

Communications Earth & Environment
orcid.org/0000-0001-7650-289X

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Senior Editor
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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of Long-term wetland biomonitoring highlights the different impacts of anthropogenically induced factors on regional macroinvertebrate diversity in Dongting Lake, China by Zhang and colleagues.

In this study, Zhang and colleagues collected long term datasets from the Dongting Lake region, and subsequently assessed the ecological status of Dongting Lake to identify major drivers responsible for the observed changes among multiple stressors derived from anthropogenic activities. Overall, this study has been well designed and the manuscript has been well organized and written. The datasets presented in this study, as well as associated analyses, are the most comprehensive that I have ever seen for the Dongting Lake region. Some major findings, such as alpha diversity changes were most influenced by land use changes 1.5 km from the sampling sites, while beta diversity was significantly affected within 50 m, have important implications for management of Dongting Lake and surrounding areas, particularly for conservation plan proposal and implementation. I suggest some edits (see below) before its publication in the journal.

1. The authors proposed three paths (paths and pathways were mixed in the manuscript, but I prefer paths) to comprehensively test the anthropogenic stressors responsible for the observed biodiversity pattern changes. I suggest that the paths should be mentioned in the abstract with results well presented.
2. Another technical issue here is multicollinearity. It is well-known that environmental factors such as P- and N-related pollutants are significantly correlated, and such a pattern is particularly true for human activity-disturbed freshwater ecosystems. Such multicollinearity can affect the downstream analysis for the identified stressors responsible for the observed biodiversity pattern changes. I suggest that the authors should test multicollinearity and specify how and why they deal with multicollinearity.
3. The final concern that I have is the direct and indirect influence. The land use changes can result in non-point source pollution pattern changes, and further lead to altered levels of pollutants (e.g. N-

and P-related) released into water bodies. So, the identified pollution-related factors are indeed derived from the change of land use patterns. I suggest that the author should either test or discuss this point in the manuscript.

Reviewer #2 (Remarks to the Author):

I enjoyed reading this work, as the writing is clear and concise, the figures are clear and in good views. I think the study can be a potential valuable contribution, but some key expressions needed to be deliberated.

In the title and abstract, you claimed that "Our results highlight the need to attenuate local land-use effects by establishing protected buffer areas to protect biodiversity in the future.". I think the varying spatial grains indeed could influence alpha and beta diversities, as many biological and ecological patterns are scale-dependent. Therefore, scale-dependent patterns are nothing related to biodiversity conservation.

Moreover, I do have some concerns with regard to the analyses.

1, why do you conduct multiple-regression analysis repeated at different sampling scales? (Line 474-Line 496)

2, how do you extract environmental values from each spatial sampling extent?

3, It is expected a buffer from a higher spatial extent will cover all points from a site from a smaller spatial extent. Therefore, I think varying sample size at different spatial extents should be influential, can you consider how to evaluate this effect (i.e., sample size or buffer size)?

4. Why is important to conduct the multi-scale analyses? Or, in other words, what is the meaning of conducting different spatial buffer analyses? You might want to determine the influencing scale determining the alpha and beta diversity of microinvertebrates, respectively. However, this might be partially due to data generation (as point 5 below), please explain.

5. The reality is that you actually conduct local sampling in a site that is at the finest scale. Therefore, you can only vary the lake-level environmental data, which might be generated from some smoothing techniques that can be artifacts.

REVIEWER(S)' COMMENTS:

Reviewer: 1

Comments:

Comment #1: *Review of Long-term wetland biomonitoring highlights the different impacts of anthropogenically induced factors on regional macroinvertebrate diversity in Dongting Lake, China by Zhang and colleagues.*

In this study, Zhang and colleagues collected long term datasets from the Dongting Lake region, and subsequently assessed the ecological status of Dongting Lake to identify major drivers responsible for the observed changes among multiple stressors derived from anthropogenic activities. Overall, this study has been well designed and the manuscript has been well organized and written. The datasets presented in this study, as well as associated analyses, are the most comprehensive that I have ever seen for the Dongting Lake region. Some major findings, such as alpha diversity changes were most influenced by land use changes 1.5 km from the sampling sites, while beta diversity was significantly affected within 50 m, have important implications for management of Dongting Lake and surrounding areas, particularly for conservation plan proposal and implementation. I suggest some edits (see below) before its publication in the journal.

RESPONSE: Thank you very much for the very broad support and positive feedback, and we have integrated all your comments to corroborate our findings and conclusions. Please find the detailed point-by-point response as follows.

Comment #2: *The authors proposed three paths (paths and pathways were mixed in the manuscript, but I prefer paths) to comprehensively test the anthropogenic stressors responsible for the observed biodiversity pattern changes. I suggest that the paths should be mentioned in the abstract with results well presented.*

RESPONSE: The suggested changes by replacing "pathways" with "paths" were made throughout the manuscript (such as in [Line 116](#)). Additionally, we have incorporated the description of the three paths into the abstract ([Lines 28 to 29](#)): "Three possible paths were hypothesized, including "land-water connection," "climate change," and "river-lake connection".".

Comment #3: *Another technical issue here is multicollinearity. It is well-known that environmental factors such as P- and N-related pollutants are significantly correlated, and such a pattern is particularly true for human activity-disturbed freshwater ecosystems. Such multicollinearity can affect the downstream analysis for the identified stressors responsible for the observed biodiversity pattern changes. I suggest that the authors should test multicollinearity and specify how and why they deal with multicollinearity.*

RESPONSE: We fully acknowledge the importance of addressing multicollinearity in our analysis, especially in the case of multivariable regression, where we employed a generalized least squares (GLS) model, as depicted in Fig S9. Before fitting the model, we conducted multicollinearity tests using two indicators, the Variance Inflation Factor (VIF) and Pearson R. Any variables with a VIF > 10 and/or |R| > 0.70 would be

excluded from the analysis. The results indicated that none of the variables, including human land-use intensity (HLU), mean annual temperature (MAT), input charge from the Yangtze River (YRQ), total nitrogen (TN), total phosphorus (TP), and algal bloom intensity (Bloom), exhibited multicollinearity. We have provided a detailed explanation of this process in **Lines 458 to 461**: *“We assessed the potential multicollinearity among the variables by conducting pairwise Pearson correlation and variance inflation factor (VIF) analyses prior to any calculations. The results showed that all absolute correlation values ($|r|$) < 0.70, and VIF < 10, indicating no multicollinearity among the variables.”*

Comment #4: *The final concern that I have is the direct and indirect influence. The land use changes can result in non-point source pollution pattern changes, and further lead to altered levels of pollutants (e.g. N- and P-related) released into water bodies. So, the identified pollution-related factors are indeed derived from the change of land use patterns. I suggest that the author should either test or discuss this point in the manuscript.*

RESPONSE: We have conducted path analysis to assess both direct and indirect effects, as illustrated in Figure 2. Our hypothesis centered on the idea that macroinvertebrate diversity could possibly be influenced by three paths, including both direct and indirect effects of land-use, climate change, and river-lake connection. Specifically, in the “land-water connection” path, we postulated that increases in human land-use intensity around the lake lead to elevated dissolved nutrient levels in the water, subsequently altering macroinvertebrate communities. We assessed the major mechanisms affecting biodiversity by conducting confirmatory path analysis based on piecewise fitting of component hierarchical linear mixed-effects models. Path analysis is a widely accepted method for examining both direct and indirect effects ^[1-4]. We have made clarifications on this method in the “Methods” section.

Lines 470 to 472 in the “Methods” section: *“Path analysis is a widely accepted method for exploring complex causal relationships and assessing direct and indirect effects in a structured and systematic way ⁵⁹.”*

Lines 278 to 286 in the “Discussion” section: *“We found that the increased human land-use intensity, including expanding agricultural and urban areas, was the dominant factor affecting the integrity of Dongting Lake. Multiple expectations of possible mechanisms, which could be categorized as direct effects, such as the toxicity of excessive nutrients, and indirect effects, such as anoxia caused by cyanobacteria blooms, have been proposed and thoroughly discussed in the field and mesocosm studies. Previous studies showed that excess nutrients lead to changes in the element stoichiometry of macroinvertebrates ³⁹, even causing the death of some species ⁴⁰ or indirectly reducing the diversity of macroinvertebrates through anoxia caused by cyanobacteria blooms ⁴¹.”*

References:

- [1] Finney, J. M. (1972). *Indirect effects in path analysis*. *Sociological Methods & Research*, 1(2), 175-186.
- [2] Wootton, J.T. (1994), *Predicting Direct and Indirect Effects: An Integrated Approach Using Experiments and Path Analysis*. *Ecology*, 75: 151-165. <https://doi.org/10.2307/1939391>.
- [3] Mysterud, A., Yoccoz, N. G., Langvatn, R., Pettorelli, N., & Stenseth, N. C. (2008). *Hierarchical path analysis of deer responses to direct and indirect effects of climate in northern forest*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1501), 2357-2366.
- [4] Lefcheck, J. S. (2016). *piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics*. *Methods in Ecology and Evolution*, 7(5), 573-579.

Reviewer: 2

Comments:

Comment #1: I enjoyed reading this work, as the writing is clear and concise, the figures are clear and in good views. I think the study can be a potential valuable contribution, but some key expressions needed to be deliberated.

In the title and abstract, you claimed that "Our results highlight the need to attenuate local land-use effects by establishing protected buffer areas to protect biodiversity in the future.". I think the varying spatial grains indeed could influence alpha and beta diversities, as many biological and ecological patterns are scale-dependent. Therefore, scale-dependent patterns are nothing related to biodiversity conservation.

Moreover, I do have some concerns with regard to the analyses.

RESPONSE: We greatly appreciate the positive recognition from the reviewer. The concept of creating land buffers with multiple sizes was based on the idea of linking aquatic and terrestrial ecosystems through the exchange of resources, particularly nutrient loadings. Freshwater ecosystems are profoundly influenced by the surrounding terrestrial ecosystems in most landscapes worldwide. Consequently, in these connected systems, a characteristic in one ecosystem resulting in an imprint on the diversity of the other ecosystem is expected, with implications for land management and conservation [1].

Currently, there are some ecological restoration measures in place that consider the “land-water connection”. However, due to the lack of theoretical frameworks and tools to address this issue within a systematic conservation planning framework, the implementation of such plans has been suboptimal [2-3]. One of the reasons for this is that the spatial extent of the impact of terrestrial disturbances on aquatic biodiversity has not yet been adequately addressed. Therefore, we have further investigated the spatial extents of “land-water connection” to provide a theoretical foundation for biodiversity conservation and ecological restoration measures considering terrestrial effects in Dongting Lake.

Besides, instead of “scale-dependent”, we would prefer the term “spatial extent”. Spatial scale can refer to the level of aggregation in geographic data, such as analyzing data at the global, regional, national, or local level. Instead, spatial extent refers to the geographic area that is encompassed by a particular dataset, defining the boundaries of the area being studied. Our analysis was based on local-scale macroinvertebrate data and only human land-use intensity was extracted from land buffers across 18 spatial extents.

We have clarified that in the Discussion: “Current restoration and convention efforts considering the "land-water connection" face challenges in implementation due to a lack of theoretical frameworks, particularly in addressing the spatial extent of terrestrial disturbances on aquatic biodiversity^{49,50}.” (Lines 338 to 341)

References:

- [1] Polis, G. A., Anderson, W. B., & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual review of ecology and systematics*, 28(1), 289-316.
- [2] Cantera, I., Coutant, O., Jézéquel, C., Decotte, J. B., Dejean, T., Iribar, A., ... & Brosse, S. (2022). Low level of anthropization linked to harsh vertebrate biodiversity declines in Amazonia. *Nature Communications*, 13(1), 3290.
- [3] Zhang, H., Mächler, E., Morsdorf, F., Niklaus, P. A., Schaepman, M. E., & Altermatt, F. (2023). A spatial fingerprint of land-water linkage of biodiversity uncovered by remote sensing and environmental DNA. *Science of the Total Environment*, 867, 161365.

Comment #2: *why do you conduct multiple-regression analysis repeated at different sampling scales? (Line 474-Line 496)*

RESPONSE: As we mentioned in **Comment #1**, the spatial extent of the impact of terrestrial disturbances on aquatic biodiversity has not yet been adequately addressed. Therefore, we have further investigated the spatial extents of “land-water connection” to provide a theoretical foundation for biodiversity conservation and ecological restoration measures considering terrestrial effects in Dongting Lake.

In practice, we extracted human land-use intensity from 18 land buffers for each sampling site across 18 spatial extents. For each sampling site and spatial extent, linear regression models were fitted between human land use and ecological responses (total nitrogen, bloom intensity, species richness, and functional redundancy) over time.

Comment #3: *how do you extract environmental values from each spatial sampling extent?*

RESPONSE: It appears that our method may not have been adequately clarified. In our spatial extent analysis, we only extracted human land-use intensity data from each specific spatial extent. We have clarified that in **Lines 488 to 500**: “Human land-use intensity was measured over multiple spatial extents. Specifically, 18 buffers around each sampling site were created at intervals of 0.05 km between 0.05 and 0.1 km, 0.1 km between 0.1 and 0.9 km, 1 km between 1 and 5 km, and 5 km between 10 and 20 km with ArcGIS 10.7. Human land-use intensities within the buffer zones were extracted, and sites with more than 80 % water in the buffer area were removed. The absolute human land-use area depends on the surface area measured within each spatial extent, making the absolute value of human land-use dependent on the spatial extent under consideration. Similarly, within each spatial extent, a buffer from a higher spatial extent covers all points from a site from a smaller spatial extent, once again making the human land-use area dependent on the area under consideration. Therefore, the human land-use intensity was calculated as a percentage the absolute human land-use area divided by the surface area.”

Comment #4: *It is expected a buffer from a higher spatial extent will cover all points*

from a site from a smaller spatial extent. Therefore, I think varying sample size at different spatial extents should be influential, can you consider how to evaluate this effect (i.e., sample size or buffer size)?

RESPONSE: Indeed, the absolute human land-use area depends on the surface area measured within each spatial extent, making the absolute value of human land-use dependent on the spatial extent under consideration. Similarly, within each spatial extent, a buffer from a higher spatial extent covers all points from a site from a smaller spatial extent, once again making the human land-use area dependent on the area under consideration. Therefore, the human land-use intensity was calculated as a percentage the absolute human land-use area divided by the surface area. (Lines 493 to 500)

Furthermore, Null models were developed to ensure that the spatial association between aquatic parameters and terrestrial land use was not a measurement artifact. Specifically, the spatial location of pixels within the Dongting Lake basin was randomly shuffled 1,000 times, and the human land-use proportion for each sampling site was extracted according to the distance buffers presented above. Then, the process applied to the "true" dataset was applied to the randomized data. (Lines 505 to 507)

Comment #5: *Why is important to conduct the multi-scale analyses? Or, in other words, what is the meaning of conducting different spatial buffer analyses? You might want to determine the influencing scale determining the alpha and beta diversity of microinvertebrates, respectively. However, this might be partially due to data generation (as point 5 below), please explain.*

RESPONSE: Please refer to the responses to **Comment#1** and **Comment#4** for details.

Comment #6: *The reality is that you actually conduct local sampling in a site that is at the finest scale. Therefore, you can only vary the lake-level environmental data, which might be generated from some smoothing techniques that can be artifacts.*

RESPONSE: We developed null models to address this issue. Please refer to the responses to **Comment#3** and **Comment#4** for details.

1st Dec 23

Dear Professor Ji,

Your manuscript titled "Long-term wetland biomonitoring highlights the different impacts of anthropogenically induced factors on regional macroinvertebrate diversity in Dongting Lake, China" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have adequately revised this manuscript. I suggest acceptance for publication.

Reviewer #2 (Remarks to the Author):

I think the authors had adequately revised the paper according to my comments. I actually did not have more comments for the authors to revise.

REVIEWER(S)' COMMENTS:

Reviewer: 1

Comments:

The authors have adequately revised this manuscript. I suggest acceptance for publication.

RESPONSE: We greatly appreciate the positive recognition from the reviewer.

Reviewer: 2

Comments:

I think the authors had adequately revised the paper according to my comments. I actually did not have more comments for the authors to revise.

RESPONSE: Thank you very much for the very broad support and positive feedback.