

Letter to the recommender

Dear recommender,

Thank you for your feedback in addition to that of the reviewers'. We strove to make the most of this chance to revise and improve our manuscript.

Notably, we added the requested information about statistic models and their significance, and now provide more context information about our study system (i.e. the distribution of environmental variables, taxa list).

Additionally, we took the chance to extent the discussion part about possible reasons for the observed varying responses among the taxonomic groups along the nutrient gradient.

Please find below (in green italics) our detailed responses to the reviewers' comments.

We hope that the improvements made to the manuscript thanks to the reviewer's remarks, now make our work suitable to be accepted by PCI Ecology.

We remain at your disposal for any further request.

*Yours sincerely,
The authors*

Revision round #1

Review by anonymous reviewer 1, 18 Mar 2024 02:50

The authors focus on the relationships between phosphorus concentration and macroinvertebrate community stoichiometry. This is a good question in freshwater systems. The authors used a large scale dataset to explore the relationships between nutrient and macroinvertebrate community stoichiometry. The statistical analysis and figures can meet the hypothesis.

1. The authors can specify where the knowledge gap is and organize the introduction part better. The authors can specify where the knowledge gap is.

Thank you for the remark. Following L. 76 we state clearly that '...not many works have addressed stoichiometric questions considering consumer communities as a whole, and no study yet has explicitly investigated community stoichiometry on a large spatial scale'. We now however specify that it seems thus worth investigating 'to close this research gap' in the next sentence (L. 80).

2. How to explain the different trend between different groups. *We extended the paragraph in the discussion that addresses the stoichiometry in Insecta and Crustacea communities by: "Hirudinea seemed to be independent towards external nutrient drivers likely, at least partly, due to a hematophagous (parasitic) feeding strategy expressed widely within this group. Opposing responses of community stoichiometry under different levels of nitrogen as observed for Gastropoda, could indicate a biochemical co-limitation of nitrogen and phosphorus (Arrigo 2005)." (L. 364)*

3. line 132, NO32- should be NO3-. *Thank you; we corrected.*

4. The authors said the community stoichiometry was calculated based on log transformed abundance multiplied by stoichiometry value. I think the log transformation should be done after the abundance multiplied by the stoichiometry value.

Thank you for the comment, indeed both approaches are possible to calculate community-weighted mean (CWM) traits – such as our community stoichiometry. Since our calculation is based on taxon values taken from a database rather than in-situ measurements of (all) individuals, we base our calculation on transformed abundances to reduce the influence of extreme abundances. This approach is broadly used, for example for macroinvertebrate traits by Guareschi et al. (2021), Peng et al. (2021) or Latli et al. (2017) and is also implemented as default for CWM in the biomonitorR R package (Laini et al. 2022).

We nevertheless repeated the analysis with log-transformation applied on community stoichiometry values (instead of the abundances prior to calculation). We find that this does not affect the general trends detected, except for (some) slopes being steeper, which is to be expected since it was not accounted for extreme abundances. Below we provide figures of this alternative calculation for the larger taxonomic groups.

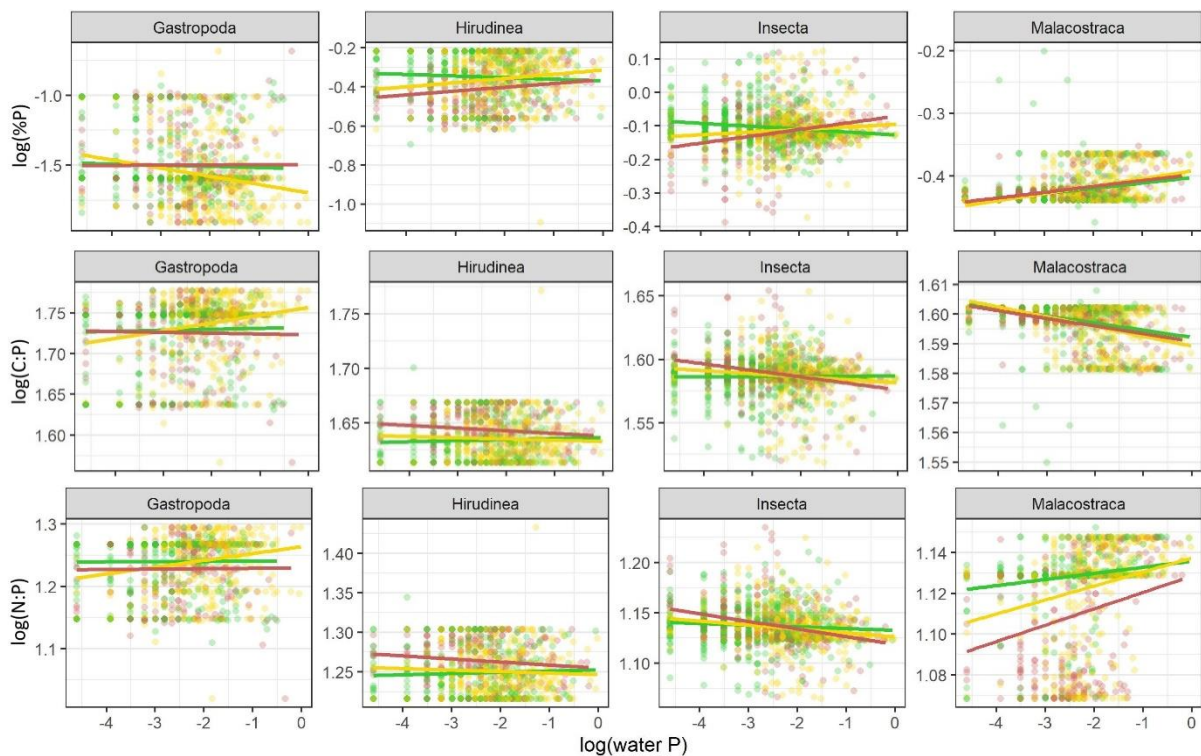


Fig 1. : Community stoichiometry within larger taxonomic groups, calculated using raw abundances and log-transformation applied after calculation.

References:

Arrigo, K. R. 2005. Marine microorganisms and global nutrient cycles. - Nature 437: 349–355.

Guareschi, S., Laini, A., England, J., Johns, T., Winter, M. and Wood, P. J. 2021. Invasive species influence macroinvertebrate biomonitoring tools and functional diversity in British rivers. - Journal of Applied Ecology 58: 135–147.

Laini, A., Guareschi, S., Bolpagni, R., Burgazzi, G., Bruno, D., Gutiérrez-Cánovas, C., Miranda, R., Mondy, C., Várбірó, G. and Cancellario, T. 2022. biomonitoR: an R package for managing ecological data and calculating biomonitoring indices. - PeerJ 10: e14183.

Latli, A., Descy, J.-P., Mondy, C. P., Floury, M., Viroux, L., Otyjacques, W., Marescaux, J., Depiereux, E., Ovidio, M., Usseglio-Polatera, P. and Kestemont, P. 2017. Long-term trends in trait structure of riverine communities facing predation risk increase and trophic resource decline. - Ecological Applications 27: 2458–2474.

Peng, F.-J., ter Braak, C. J. F., Rico, A. and Van den Brink, P. J. 2021. Double constrained ordination for assessing biological trait responses to multiple stressors: A case study with benthic macroinvertebrate communities. - Science of The Total Environment 754: 142171.

5. In all scatter plot figures, the authors should add information about the sample size, the r value and P value.

The sample size for each category of water level N is identical for each panel and was already provided in the figure legend. As a compromise between providing the information and remaining readability/clarity of the figures, we added r^2 values of the linear models to the figures and provide exact p -values and r^2 values in the results tables.

6. As the community stoichiometry was mainly related to the community abundance, the authors should add figures about the community abundance and the functional feeding groups. *As part of the analysis our main manuscript contains figures showing the proportions of taxonomic and feeding groups along the nutrient gradients, which are also discussed in the discussion. Please note that “A taxa list including the feeding group classification is [now] available in S10.” (L. 178).*

7. The authors should also add information about the dynamics of total carbon, total nitrogen, and total phosphorus in water. *Please note that “Distributions of water N and P concentrations are [now] available in S1.” (L. 138). Unfortunately, we do not have POC (or other carbon measure) data for the dataset.*

8. I think to think about relationship between water stoichiometry and community stoichiometry maybe better.

Thank you for the comment. Indeed, we considered this option in the beginning, but then realised that we did not have reliable exact values of nitrogen and phosphorus due to detection limits (as discussed in the MM). We thus decided to integrate water nitrogen level as categorical value N , which we could derive with confidence.

Review by Jun Zuo, 21 Mar 2024 07:11

Beck et al. used data from large-scale field sampling of sites varying in nutrient levels, which were helpful to reveal the link between the community stoichiometry and nutrient. There are several points that needs to be clarify.

A taxon with fast growth rate in P-enriched is not equal to one with high body %P. Back et al 200, potential growth rates, higher p content should have faster growth
The water P would directly affect the phytoplankton (primary producer), rather than

consumers, e.g. stream macroinvertebrates. The macroinvertebrates might prefer to preying the taxa with similar stoichiometry. In this manner, are there no linear relationship between water P and macroinvertebrates? As the author stated that although they can have a relatively high body phosphorus content (Cross et al., 2003; González et al., 2011), their prey are stoichiometrically more stable than the resources of primary consumers making prey identity probably more important. (Line 381-384)

Thank you for your comment. You are right, prey identity likely is more important for preying taxa. However, herbivorous and detritivorous taxa feed on primary resources or detritus whose nutrient content is directly influenced by nutrient concentrations in the environment as we clearly addressed in the introduction for example stating that “nutrient enrichment lowers C:nutrient ratios of primary producers, thus increasing their quality for herbivore consumers” (L. 55). We aimed to address such differences between primary and secondary consumers in our second hypothesis, investigating community stoichiometry responses also feeding-group-wise. As stated, “ The community stoichiometry of primary consumers (detritivores, herbivores and omnivores) should be more strongly affected than that of secondary consumers (predators), whose resources are stoichiometrically more stable.”(L. 91)

It is better to show the R2 and P value in all multiple linear models.

Please note that we added r2 values to the figures and provide exact p-values and r2 values in the result tables, which seems a good compromise between providing the information and remaining the readability of the figures.

Line 132-134 The nitrate concentration in stream water can be higher than 25 mg/L. I really doubt this data. In my opinion, the total nitrogen concentration is often less than 10 mg/L.

Please note that “Distributions of water N and P concentrations are [now] available in S1.” (L. 138). The monitored sites do not only include good quality sites (indeed characterised by low concentrations of nitrogen), but also ‘polluted’ sites. Nevertheless, sites of ‘good’ or ‘intermediate’ nitrogen level make the majority of SSE in our dataset as also indicated in the figure descriptions.

Line 159-160 Author selected taxa for which the stoichiometric information was available. Which taxa? Is there a database or a reference for specific taxa stoichiometric information?

As stated for example in L. 150 (and briefly L. 100), “Stoichiometric information, i.e. major nutrients (%C, %N, %P) and the corresponding molar ratios (C:N, C:P, N:P) [...] was obtained from a database (Beck et al., 2022).” Please note that “A taxa list including the feeding group classification is [now] available in S10.” (L. 178)

Line 174-180 an affinity > 0.5 is arbitrary. What is the reasoning behind this threshold?

As we added now, an affinity of >0.5 means ‘the dominant share’ (L. 173) of a taxon’s dominant feeding resource. Also, we classified taxa without clear preference as ‘omnivore’. Since there are almost no taxa with a strict carnivorous or herbivorous diet (see the trait database in Tachet et al., 2010), we decided that this threshold would well represent general feeding behaviours.

Review by Thomas Guillemaud, 20 Feb 2024 08:45

Data with metadata and script are accessible, a readme file is present. The script is well commented and understandable. It runs well on the data and produce the same results as in the article.

1- Can we get the data and script from the links indicated in the submission form or from the article itself? Yes

2- Is there a readme file. Yes

3- Are there metadata for the data and comments for the scripts? Yes

4- Are the readme, and data files understandable by a normal reader? Yes

5- Do the scripts run on the data? Yes

6- Are the results the same as in the paper? Yes

The Email exchange I had with the authors is in the attached pdf file.