

Dear Pierre Labadie,

Please find attached the manuscript that my co-authors and I would like to publish in *PCI Ecotoxicology and Environmental Toxicology*. We would like to thank you for the opportunity to resubmit the manuscript and the reviewers for their comments, which have helped to improve it.

We modified the manuscript in line with all the reviewers' comments. Please find below a point-by-point response to these comments. Our responses are highlighted in grey as are our changes to the text.

The data and the scripts are available on a depository (In.Do.RES) but they are under embargo until publication. The article and the supplementary material are available on BioRxiv. The DOI, conflict of interest disclosure and funding are given at the end of the article.

We hope that we have fully addressed all the changes requested by the reviewers. We look forward to hearing from you in the near future.

Yours sincerely,

Florence D. Hulot, on behalf on my co-authors

Review by anonymous reviewer 1, 08 Aug 2024 13:41

The revised manuscript submitted by Florence D. Hulot and co-workers has been conscientiously reworked. The co-authors have provided coherent responses to the main reviewers' comments and proposals. They significantly clarified rather confusing points of the previous version of the manuscript, and simplified the presentation of the results by removing certain superfluous or questionable analyses from the main manuscript. An effort has been devoted to (1) present a more comprehensive description of the methodological and statistical design, and (2) better structure the results and discussion sections. All these modifications have resulted in a substantially improved manuscript.

Nevertheless, I still have some comments and suggestions, both on the authors' responses to reviewers' comments after the first round of review (part A, in bold) and on the revised version of the manuscript (part B).

A) Comments on the authors' responses to reviewer 1 comments

1. Reviewer 1 comment (1st round):

Lines 49-58: The authors wisely emphasise the importance of pond size as an element of vulnerability to anthropogenic impacts, but curiously do not include this size among the environmental variables that could explain between-ponds variations in the response of taxonomic structure or taxonomic biodiversity of invertebrate assemblages to anthropogenic pressures (e.g. in the MFA). Why not?

Authors' response: We have added the surfaces in the Materials and Methods section (lines 132-133). These surfaces were estimated on a single date and do not correspond to the actual surface areas of the ponds during sampling, which can be quite variable. For this reason, it is not relevant to include them in analyses

Reviewer 1 comment (2nd round):

Agreed ... but just because the size of the ponds was not measured at each sampling date does not mean that it is not an important parameter driving the structure and composition of the benthic macroinvertebrate assemblages, especially if this size varied greatly from one date to the next.

Authors' response (2nd round):

We agree. We tried to measure the surface area of ponds on Sentinel 2 images, but as some were under forest cover or a little shaded, it was not possible to do this for all the ponds. We have added a warning sentence on lines 263-265: “. Although the surface area of ponds is important, as pointed out in the introduction, it could not be included as a factor in the analyses because we only have a single measurement.” In addition, we corrected the size of the biggest pond (from 5108 to 828 m²) as it has been initially calculated in a year when the water level was abnormally high (line 133).

We also calculated the ratio pond surface/buffer surface and, in percentage, it varies from 0.19 to 2.29, which stays quite low.

2. Reviewer 1 comment (1st round):

Lines 64-66: But probably largely depending on their diversity in aquatic (meso)habitats? Has the within-pond diversity of (meso)habitats (sensu Armitage & Pardo, 1995) been considered in the study to potentially explain variation in invertebrate assemblage in relation to anthropogenic impacts?

Authors' response: We agree on the importance of meso-habitat diversity, which is why we sampled two points in each pond. However, our aim was to study the effects of contaminants and to monitor a large number of them, taking into account their diversity. Given the large number of analyses, we pooled samples within each pond. We did not design a protocol to study meso-habitat diversity, as this was not our objective.

Reviewer 1 comment (2nd round):

Agreed ... However, contaminants can have indirect effects on the structure and composition of benthic macroinvertebrate assemblages, by modifying the mosaic of benthic mesohabitats (e.g., by influencing the composition of algal or macrophytic communities, in variable ways depending on the composition of the contaminant cocktail).

Authors' response (2nd round):

We do agree that indirect effects of contaminants on the structure and composition of benthic macroinvertebrate assemblages, acting through modification of the mosaic of benthic mesohabitats are possible and very interesting. Our study is conceived as a first examination of the effects of a large number of contaminants on these assemblage, we therefore focused on the direct effects. But it will be very interesting to design a follow-up study on these ecosystems to assess these indirect effects.

3. Reviewer 1 comment (1st round):

Lines 127-129: Why a buffer zone of 100 m radius has been uniformly considered to describe the proportion of agricultural and urbanised land around each pond, whatever the size of the pond, given the wide range of sizes of the ponds selected? This point should be justified a little more. First, the effects of adjacent land use on both water quality and biotic assemblages have already been considered as strongest at larger distances (e.g. between 200 m and 4000 m; see Houlihan & Findlay, 2003; 2004; Houlihan et al., 2006). Second, would it not have been more appropriate to adapt the size of the buffer zone considered, taking into account the size of the pond, as was done - for example - by Labat & Usseglio-Polatera (2023)?

Authors' response: To justify our choice, we added the following sentence (lines 135-136): "As the aim was to study the effect on water quality of land use in the vicinity of ponds in a fairly fragmented landscape, we used a short-radius buffer."

Reviewer 1 comment (2nd round):

The additional information provided does not constitute a justification of the method used, given the question posed; I would have much preferred the authors to indicate why they chose to adopt an identical buffer size, whatever the surface area of the ponds.

Authors' response (2nd round):

In our opinion, it is very difficult to design a buffer zone that is adapted to the surface area of the pond, as this can vary over time (filling in) and with the seasons. Many studies use buffer zones with a constant radius, whatever the surface area of the pond. It seems that the buffer zone is between 30 and 200 m (Oertli & Frossard 2013). To find the right buffer zone for the waterhole, you need to think in terms of a catchment or sub-catchment, taking into account various parameters such as topography, soil type, and so on.

4. Reviewer 1 comment (1st round):

Lines 145-147: The field campaigns appear to have been considered homogeneously. Why didn't the authors try to deconvolute the seasonal effect (June vs. September) from the interannual effect (2016 vs. 2017)?

Authors' response: Our focus is on contaminants and we measured a lot of them twice a year during two years. The number of repetition (2) is not high enough to meaningfully deconvolute seasons and years.

Reviewer 1 comment (2nd round):

I don't agree. Given the experimental design adopted, it would have been possible to highlight any seasonal or inter-annual effects. If the environment of the ponds is essentially agricultural, it can be assumed that phytosanitary products are applied according to the vegetative cycle of the cultivated species, and therefore the intensity of contamination can be extremely variable from one season (or one year) to the next.

Authors' response (2nd round):

Given the small number of years (2) and seasons (2), it is not possible to carry out a temporal analysis. However, we carried out a correspondence analysis with the presence/absence data (as in Benedetti et al 2019). Then we recovered the scores of the ponds on axes 1 and 2 and tested them for season * year effects. The CA shows that the axes explain very little and the anova shows a season effect ($p < 0.0001$) on axis 2 which explains 4.8% of the variance. As the results are not robust, we conclude that there is no season or year effect on the assemblages. This analysis has been added to the appendix with a warning in the text (lines 292-293).

5. Reviewer 1 comment (1st round):

Lines 180-183 and tables S4/S5: .../... Moreover, the authors have indicated that "when it was not possible to link the different stages (larvae, nymph, adult) to the same species, they were assigned to different morphotaxa". In my opinion, this second major decision adds a further level of uncertainty to the quantification of invertebrate assemblage diversity. An indirect effect is also that the authors implicitly give more weight in the data - and therefore in the analyses - to "poorly identified" taxa, insofar as they may constitute several morphotaxa (corresponding to several stages of development or because they may contribute to taxa of

different systematic levels - e.g. Diptera/Chironomidae /Chironomini/Chironomus) to the detriment of taxa “reliably identified” at the specific level (e.g. *Hydrometra stagnorum*, *Ochthebius minutus* or *Limnoxenus niger* in Table S4 and S5), which is not ecologically justified. What impact did these authors' choices have on their ability to interpret "taxonomic richness" or "taxonomic diversity" based on such morphotaxa in ecological terms? This crucial point needs at least to be deeply discussed.

Authors' response: *We agree that this has an impact on results and analyses. As our objective is to compare ponds at 4 dates, we believe that this way of considering specimens does not alter our analysis, as we have always kept the same methodology. We do not compare diversity indices with ponds other than those in our study. In addition, we have kept the presence[1]absence analyses in the main text and discuss them. Analyses with abundances, other than diversity indices, may contribute to increased bias due to over-represented groups; they are now in the appendices. We have added a warning lines in the Material and methods section (lines 264 – 266).*

Reviewer 1 comment (2nd round):

I don't completely agree with your argument. Just because the same methodology has been used on all the sites does not mean that the communities associated with these sites will be affected in the same way by the methodological choices made regarding the systematic description of taxa. This might be the case if the taxonomic structures of the benthic invertebrate assemblages at the sites were similar. If certain sites have, for example, a higher proportion of Chironomidae (or organisms that are difficult to identify at a specific level) in their community composition, they will potentially be more affected than others by the choices made in terms of identification level.

Authors' response (2nd round):

We agree that the proportions can vary between ponds. However, we believe that this does not affect the differences between pools, only the size of the differences. The alternative would be to carry out the analyses at family level, but that would mean losing a lot of information. Our choice to consider all the morphotaxa is fairly classic (Florencio et al., *Insect Conservation and Diversity*, 10.1111/icad.12029; Hill et al. 2015, *Hydrobiologia*, 10.1007/s10750-015-2328-8; Hill et al. 2016, *Environmental Monitoring and Assessment*, 10.1007/s10661-016-5178-6).

6. Reviewer 1 comment (1st round):

Last point on the taxonomic list: I'm not sure that Daphniidae (three morphotaxa in table S4), ostracods (six morphotaxa of the Podocopida order in table S4) and copepods (two morphotaxa) can be considered as “macro” invertebrates, and have been efficiently sampled with a 1 mm mesh size pond-net.

Authors' response: *Indeed, they are not macro-invertebrates and we redid all the analyses without considering taxa (Daphniidae, ostracods and copepods).*

Reviewer 1 comment (2nd round):

OK. The decision to repeat the analyses without these taxa is a wise one.

7. Reviewer 1 comment (1st round):

Lines 230-231 and table 1: I'm not sure to clearly understand which kind of ANOVA has been performed: Are the results provided in table 1 correspond to (i) "one-way ANOVAs" independently testing the "pond" effect and the "field session" effect (but two numbers of degrees of freedom should have been provided by ANOVA) or (ii) "twoway ANOVAs without replication" simultaneously testing both effects (but three numbers of degrees of freedom should have been provided by ANOVA).

Authors' response: *We did two way ANOVAs with additional effects of "pond" and "field session". It is now specified on line 270.*

Reviewer 1 comment (2nd round):

Thank you for this additional information.

8. Reviewer 1 comment (1st round):

Lines 239-241: The authors have explored two different analysis strategies for measuring pond dissimilarity based either on taxon abundances (with Hellinger coefficient) or occurrences (with Jaccard coefficient). After reading the MS, I wonder whether it is really necessary to present both approaches in the main manuscript. Examine the possibility to move the presentation of one approach in the Supplementary Material.

Authors' response: *We moved the approach based on abundances in Supplementary section.*

Reviewer 1 comment (2nd round):

Thank you for taking my comment on board. This choice simplifies the presentation of the results.

9. Reviewer 1 comment (1st round):

Lines 250-255 and 265-276:

.../... Consequently, is MFA objectively adapted to the analysis of the data at the scale of the sampling unit (i.e. pond x field session)?

Authors' response: *Thank you for your comment, as we couldn't understand why the ellipses were flattened! As the land use did not change between sessions, we preferred to withdraw the analysis. We ran a linear mixed-effect model as specified on lines 297-299.*

Reviewer 1 comment (2nd round):

I agree. I also think this is a good choice.

10. Reviewer 1 comment (1st round):

Lines 475-482: It seems difficult for authors to avoid discussing the relevance of their assessment of alpha and beta diversity, given the choices made in defining morphotaxa (see my comments on Lines 180-183 and tables S4/S5).

Authors' response: *Our comparisons focus on ponds as a function of pollutant concentrations. Our morphotaxa definitions (lines 203-204) are the same for the 4 sampling sessions. So it does not interfere with the analyses.*

Reviewer 1 comment (2nd round):

For me, it's not so obvious (see my comment 5).

Authors' response (2nd round):

See response comment 5.

11. Reviewer 1 comment (1st round):

Non-capture of pollution-sensitive or rare taxa is also worth discussing. Which taxa were expected in these ponds? Was the sampling method (without scraping the bottom substrate) really adapted to their capture?

Authors' response: *We did not expect specific taxa and we observed benthic organisms. The sampling method, with infinite signs, resuspended sediments and benthic individuals that were collected in the sampling net.*

Reviewer 1 comment (2nd round):

I'm not sure I understand the argument.

Authors' response (2nd round):

The sampling method, with repeated movements over the sediment, creates a suction effect, which is appropriate because we collected organisms typical of the benthos, such as adult bivalves. Our approach is naive because there are few studies on peri-urban environments and we don't know what species we can expect. If the organisms present were not caught despite 4 sampling sessions, it is because they are really rare and that we were at the plateau of the rarefaction curve. We have completed the sentence line 170 by adding "thanks to the upward current created by the "infinity" movement".

12. Reviewer 1 comment (1st round):

Lines 531-560: Given the variety of pond uses, the size of the pond was undoubtedly an important factor to consider (local uses are likely to have more or less impact depending on their size).

Authors' response: *We did not analyse the effect of pond size as we have only one measure, i.e. we did not measure the size of the pond at each sampling session. Moreover, we have one small pond, one big pond and the others are of roughly the same size.*

Reviewer 1 comment (2nd round):

I understand that the size of the ponds was not measured at each sampling date. Nonetheless, even if the average size of the ponds is relatively similar, seasonal variations in size may be more or less significant depending on the pond, and if we consider that toxic inputs are relatively constant over the year, the toxic impact on native fauna may be more or less significant depending on the overall volume of water in the pond at the sampling date.

Authors' response (2nd round):

See response comment 3.

B) Comments and edits on the revised version of the manuscript

Line numbers correspond to the revised version of the MS (with “hidden” modifications)

Lines 28-29: There seems to be a problem with the second part of this sentence, which is not clearly understandable. Please, rewrite.

- Done

Line 32: The following wording is perhaps clearer: « with the exception of the proportion of agricultural land in the vicinity of the pond on equitability”

- Done

Lines 37-38: Change in « ... are shaped by sediment levels of pharmaceuticals, water conductivity and ammonium concentration.”

- Done

Line 120: Change « make a high contribution” in “highly contribute”.

- Done

Line 133: Add a space between “566” and “m2”.

- Done

Line 137: Change « buffer » in “buffer zone”.

- Done

Lines 192-193: Not very clear: the wording of the sentence suggests that there are two sediment sampling phases: "sediment" (?) and "surface sediment" (?). Is this really the case? This needs to be clarified.

- We changed the sentence in: "Sediment sampling was performed with the same device used to collect the water samples." (lines 193-194)

Lines 198-199: "Morphological identification" or "identification on morphological criteria" (are probably better than "optical identification"). The following information (on the type of stereomicroscope used) already indicates that the determination was "optical".

- "optical identification" changed to "morphological identification"

Line 205: Change « larvae » in "larva".

- Done

Line 217: « farming » or « farms »?

- Changed "farming" to "farms"

Line 222: alternative: "For the water samples, the following measurements were taken directly on site using probes: ..."

- Done

Line 224: « a » or « one » ?

- within one day

Line 267 : Which evenness index has been calculated: the Pielou index? The Hurlbert index? Another index? Please specify.

- Pielou evenness - corrected

Lines 275-278: These two sentences could be merged in a single one: "This function partitions total beta diversity into two additive components, turnover and nestedness, which reflect species replacement and species richness difference respectively (Baselga et al. 2020)."

- Done

Lines 281 and 287: Change « Caceres » (or "De Careres") in « De Cáceres ».

- Done

Line 297: Change « tested the proportion of urban, forested and agricultural areas on ... » in "tested the effects of the proportions of urban, forested and agricultural areas on ..."

- Done

Line 298: Change “equitabilty” in “equitability”.

- Done

Line 309: Change « and » in « from”.

- Done

Line 313: Change « following analysis » in “following analyses”.

- Done

Lines 319-321: Change the comma in a colon after “for water” and “for sediments”.

- Done

Line 331: Change « that we tested » in “which we have tested”.

- Done

Line 340: Change « diversity » in « richness”.

- Done

Lines 348, 452 and throughout the MS: Change « eveness » in « evenness ».

- Done

Line 356: Change “makes” in « has » or « exhibits »?

- Done

Line 363: “between » or « among »?

- Corrected to “among”

Line 368: Delete the comma after “macrostoma”.

- Done

Lines 384-385: A bit strange « to be decomposed “into” ... only a “single” canonical axis. Please, rewrite.

- Replaced by “is reduced to” one significant canonical axis

Line 386: « high » concentrations?

- Added “high”

Line 394: associated to “high values” of both variables.

- Added “high values”

Line 404: Change “It” in “it”.

- Done

Line 406: « annual » or « seasonal »?

- An annual effect. We changed our protocol between the 2 years and annual changes can mask the effects of the protocol change.

Line 407: Add “taxonomic” before “levels”.

- Added

Line 410: Not so much, insofar as the taxa for which numerous 'morphological forms' have been defined are not evenly distributed ... and their distribution (e.g. for the Chironomidae) is not independent of the ecological status of the pond (since this is a taxonomic group considered to be highly pollutant-tolerant). (see my comment 5).

Lines 411-414: I don't necessarily share your interpretation of the results of these papers. Llopis-Belenguer et al (2023) have indeed studied the impact of the systematic level of parasite identification in the case of host-parasite relationships for simulated networks of fishes and metazoan parasites of limited size. However, the 'taxonomic resolution' effect (of the parasite) was systematically significant, whatever the community descriptor used (cf. Table 4 in the cited paper). Moreover, one of the major authors' conclusions is that studies should avoid applying bipartite network analyses to communities with low sampling effort or taxonomic resolution (cf. last sentence on page 10/14 of the cited paper).

In the case of the article by Renaud et al (2020), who worked on "plant-pollinator" relationships, the conclusions are fairly similar: the raw values of most of the indices characterising the networks are significantly impacted by a change in the level of taxonomic identification (cf. table 2 (p. 3252) and figure 1 (p. 3253) of the article cited) and only the standardization of index values measuring nestedness with the Z-score, can reduce this taxonomic resolution effect for three indices.

Lines 432/433: Change « species » in « taxa” (two times).

- Done

Line 436: To be homogeneous, change « 47 » in « 47.0 »

- Done

Lines 453-454: This result is not necessarily surprising insofar as the macroinvertebrate taxa considered to be the most sensitive to pollution are rheophilic taxa, which are much more likely to be found in rivers (if possible, with a steep gradient, limiting the risks of deoxygenation), than in ponds.

- We agree.

Lines 461-462: « is the most structuring parameter” (?)

- We changed the sentence “The concentration of pharmaceuticals in sediment and water conductivity are the most structuring parameters.” In “The concentration of pharmaceuticals in sediment and water conductivity are the most structuring parameters of macroinvertebrate assemblages.” (lines 486-487)

Line 463: Change « reveal » in « reveals”.

- Done

Line 466: Change the comma (after “ponds”) in point, and starts the next sentence with “pesticides” (after capitalizing the first letter).

- Done

Line 477: Delete one of the final point.

- Done

Lines 484 and 486: Change “Annelid” in « annelid »

- Done

Line 494: Delete one “with”.

- Done

Line 642: Change « De Caceres » in « De Cáceres »

- Done

Review by anonymous reviewer 2, 02 Aug 2024 08:45

Following the corrections made by the authors, the manuscript was really improved. There are just minor points left to correct

Line 25-26 : check this sentence

- we've added numbers [(1), (2)] to highlight the 2 objectives

Line 34 : Remove one "and"

- Done

Line 508 & 549: medical centre or medical center ?

- Corrected

Line 519 : Remove one "with"

- Done