Peer Community In Ecotoxicology & Environmental Chemistry

Integrating chemical and biological assessments to understand the impact of pollutants on freshwater biodiversity in model systems such as peri-urban ponds

Pierre Labadie *based on peer reviews by* ***Aurélie GOUTTE*** *and 2 anonymous reviewers*

Florence D. Hulot, Christophe Hanot, Sylvie Nélieu, Isabelle Lamy, Sara Karolak, Ghislaine Delarue, Emmanuelle Baudry (2024) Do macroinvertebrate abundance and community structure depend on the quality of ponds located in peri-urban areas? bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://hal.science/hal-04850220v1

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Ponds, as small freshwater ecosystems, are particularly vulnerable due to their limited size. Yet they are often overlooked in research, possibly because they are considered less important (Biggs et al., 2017). Shallow water bodies support higher biodiversity than larger aquatic ecosystems. Peri-urban areas, characterized by a blend of agricultural and urban land uses, are dynamic and constantly evolving landscapes with diverse activities and stakeholders (Zoomers et al., 2017); as such, they are referred to as ”restless landscapes” or zones of continual transformation (Zoomers et al., 2017). They often harbor neglected ecosystems, and despite their ecological importance, ponds and wetlands in peri-urban areas remain relatively underexplored (Wanek et al., 2021). Furthermore, these areas may experience increased contaminant inputs, which are regarded as one of the 12 major threats to freshwater biodiversity (Reid et al., 2019).

In this context, Hulot et al. (2025) monitored 12 peri-urban ponds in the Île-de-France region (near Paris, France) to investigate the relationships between land use, pollutant concentrations in water and sediment, and macroinvertebrate distribution. The originality of this work lies in its multidisciplinary and integrated approach, combining ecological and chemical analyses. While assessing agricultural, urban, grassland, and forest landscapes surrounding each pond, this study aimed to understand how contaminants constrain macroinvertebrate

communities. The authors hypothesized that i) ponds in grassland and forest environments support higher local diversity than those in agricultural or urban areas, ii) rare and pollution-sensitive species significantly contribute to regional diversity, and iii) contaminants in water and sediment influence the distribution of macroinvertebrate morphotaxa.

This study provides numerous novel results. Specifically, it demonstrates that fluctuations in morphotaxa composition are predominantly driven by species replacement rather than by disparities in species richness. This pattern was largely attributed to the high prevalence of pollutant-tolerant species in certain ponds. In addition, community compositions appeared to be influenced by sediment levels of pharmaceuticals, water conductivity, and ammonium concentrations. In summary, ponds located in peri-urban areas are subject to a range of human-induced disturbances, and these results suggest that these disturbances lead to chronic and varied contamination, which in turn affects the composition of morphotaxa communities.

These findings establish a clear connection between local pollution and ecological composition, a crucial aspect for effective conservation and restoration efforts on peri-urban ponds.

References:

Biggs, J., S. von Fumetti, Kelly-Quinn M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. Hydrobiologia 793(1): 3-39 625 doi:10.1007/s10750-016-3007-0

Hulot, F.D., Hanot, C., Nélieu, S., Lamy, I., Karolak, S., Delarue, G., Baudry E., (2024) Do macroinvertebrate abundance and community structure depend on the quality of ponds located in peri-urban areas? ver.3 peer-reviewed and recommended by PCI Ecotoxicology and Environmental Chemistry https://hal.science/hal-04850220v1

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. J. Johnson, 712 K. A. Kidd, T. J. MacCormack, J. D. Olden, S. J. Ormerod, J. P. Smol, W. W. Taylor, K. Tockner, J. C. Vermaire, D. Dudgeon, Cooke, S. J. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94(3):849-873. doi:10.1111/brv.12480

Wanek, A., C. L. M. Hargiss, J. Norland, Ellingson, N. 2021. Assessment of water quality in ponds across the rural, peri-urban, and urban gradient. Environmental Monitoring and Assessment 193: 694. doi:10.1007/s10661-021-09471-7

Zoomers, A., F. van Noorloos, K. Otsuki, G. Steel, van Westen, G. 2017. The Rush for Land in anUrbanizing World: From Land Grabbing Toward Developing Safe, Resilient, and Sustainable Cities and Landscapes. World Dev 92:242-252 doi:10.1016/j.worlddev.2016.11.016

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.10.20.563281
Version of the preprint: 2

Authors' reply, 13 December 2024

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Decision by Pierre Labadie, posted 08 August 2024, validated 12 August 2024

Dear Dr. Hulot,

We have now received two reviews of your revised manuscript. I will be looking forward to receive a another revised version of your manuscript following major revisions, including a point-by-point reply to each comment and suggestion made by the reviewers.

Regards,

Pierre Labadie

Recommender for PCI EcotoxEnvChem

Reviewed by anonymous reviewer 1, 08 August 2024

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.

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).

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 Houlalan & Findlay, 2003; 2004; Houlalan 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.

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.

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.

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).

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.

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.

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.

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"

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

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

Line 133: Add a space between "566" and "m2".

Line 137: Change « buffer » in "buffer zone".

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.

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".

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

Line 217: « farming » or « farms"?

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

Line 224: « a » or « one » ?

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

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)."

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

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 …"

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

Line 309: Change « and » in « from”.

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

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

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

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

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

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

Line 363: “between » or « among »?

Line 368: Delete the comma after “macrostoma”.

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

Line 386: « high » concentrations?

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

Line 404: Change “It” in “it”.

Line 406: « annual » or « seasonal »?

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

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).

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

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.

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

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

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

Line 477: Delete one of the final point.

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

Line 494: Delete one “with”.

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

Reviewed by anonymous reviewer 2, 02 August 2024

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

Line 34 : Remove one ”and”

Line 508 & 549 : medical centre or medical center ?

Line 519 : Remove one ”with”

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.10.20.563281

Version of the preprint: 1

Authors' reply, 09 July 2024

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Decision by Pierre Labadie, posted 05 February 2024, validated 06 February 2024

Revision needed for manuscript #142

Dear Dr. Hulot,

We have now received three reviews of your manuscript. I will be looking forward to receive a revised version of your manuscript following major revisions, including a point-by-point reply to each comment and suggestion made by the reviewers.

Regards,

Pierre Labadie

Recommender for PCI EcotoxEnvChem

Reviewed by anonymous reviewer 1, 03 January 2024

General comments:

The authors aim at examining the effects of various classes of micropollutants (e.g. trace elements, pharmaceuticals, pesticides, PAH) from urban and/or agricultural watersheds on the abundance and taxonomic structure of benthic macroinvertebrate assemblages of peri-urban ponds based on a 2 year-survey.

For this purpose, the authors set up various inferential and descriptive statistical analyses relating the presence/absence or abundance of taxa and taxonomic diversity metrics (i.e. alpha & beta diversities) to contaminant concentrations in the water and sediment and/or to a description of land use in the immediate vicinity (i.e. a radius of 100 m) of 12 ponds of various size (64 – 5,108 m2).

This article is interesting for the scientific community. The authors put a lot of effort into the manuscript, which is clearly written and well-structured. The core objectives are attractive; tested hypotheses are clearly defined (lines 110-114) and the information flow makes the paper a quite attractive piece of work. The literature review is comprehensive and may be useful to both ecologists and ecotoxicologists. The “material and methods” section is presented in a rather concise and reproducible way, but some major methodological choices – and their limits - need to be more deeply discussed (e.g. the definition of morphotaxa, the different sampling strategies adopted). The statistical design seems globally sound (even if not explicit enough for several steps; see my “main comments” below). The results are rather clearly described. Perspectives could be more developed; e.g. why not exploring a more functional approach based on “species traits”? Note also

that a biotic index, based on macroinvertebrates (BECOME-I#15) has been recently and specifically developed for small standing waters in mainland France, and could be used as supplementary variable in some of the analyses (Labat & Usseglio-Polatera, 2023; table 4, p. 10; reference provided below).

I have detailed my main concerns in the following "main comments".

Main comments:

Abstract:

The abstract is rather concise and clearly present the main objectives (exploring local and regional macroinvertebrate spatiotemporal diversity and understanding the effects of contaminants on the abundance and structure of pond invertebrate assemblages) and the main findings of the study.

Introduction:

The introduction is quite interesting and correctly structured.

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?

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?

Cited reference:

Armitage P.D. & Pardo I. 1995. Impact assessment of regulation at the reach level using macroinvertebrate information from mesohabitats. *Regulated Rivers: Research & Management*, 10, 147-158. https://doi.org/10.1002/rrr.3450100210

Lines 67-81: While contaminants are clearly one of the main threats to small stagnant waters, the physical degradation of littoral habitats can also be a major source of alteration to invertebrate assemblages (cf. some of the sources of degradation of ponds – e.g. trampling, tractor washing directly in the pond, etc - described in the "characteristics of the peri-urban environment" sub-section of the discussion; pp. 531-548).

Material & methods:

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 Houlalan & Findlay, 2003; 2004; Houlalan 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)?

Cited references:

Houlahan J.E., Findlay C.S. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences, 60, 1078–1094. https://doi.org/10.1139/f03-095

Houlahan J.E., Findlay C.S. 2004. Estimating the 'critical' distance at which adjacent landuse degrades wetland water and sediment quality. Landscape Ecology 19, 677–690. https://doi.org/10.1023/B:LAND.0000042912.87067.35

Houlahan J.E., Keddy P.A., Makkay K., Findlay C.S. 2006. The effects of adjacent land use on wetland species richness and community composition. Wetlands 26, 79–96. https://doi.org/10.1672/0277-5212(2006)26[79:TEOALU]2.0.CO;2

Labat F., Usseglio-Polatera P. 2023. A new bioassessment multimetric index (BECOME) and diagnostic tool (BECOMEd) for small standing waters. Ecological Indicators, 154, 110831. https://doi.org/10.1016/j.ecolind.2023.110831

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)?

Lines 159-166: It is rather strange to change the invertebrate sorting strategy so radically in the middle of the sampling plan. Have the authors examined the potential impact of such a modification on the resulting faunal list, insofar as it may constitute a potential confounding factor for robust identification of interannual variation in assemblage structure.

Lines 166-168: There is something inconsistent in the presentation of the number of samples collected: "12 ponds x 2 points x 2 years x 2 seasons, minus 6 cases" equals 90 samples (and not 44 as indicated in the MS). Even if the invertebrates collected at the two sampling points of a given pond have been pooled (as indicated in lines 223-224), the resulting number of samples does not correspond to 44. Please, explain more clearly what has been done.

Lines 180-183 and tables S4/S5: I am really embarrassed by the definition of "morphotaxa" in this document. As indicated by the authors, organisms that could not be identified at the species level were identified at a higher taxonomic level, while adding a numerical suffix when more than one species was present (e.g., Microvelia sp.2)." I understand the process and validate it if applied at given – homogeneous - taxonomic level (e.g. the genus level, as for the example provided). However, this process has been applied by the authors at different nested levels of the systematics. For example (cf. Table S4), for the dipteran family "Chironomidae", nine morphotaxa have been defined at the "family" level (Chironomidae_01 to Chironomidae-11 in table S4), six morphotaxa have been defined at the Chironomini or Tanytarsini "tribe" level (two tribes within the Chironomidae family; Chironomini_01 to Chironomini_04 and Tanytarsini_01 to Tanytarsini_02) and six morphotaxa at the Chironomus "genus" level (one of the genera of the Chironomini tribe; Chironomus_sp._01 to Chironomus_sp._06). Moreover, in this family, additional morphotaxa have been also defined at the "sub-family" level (e.g. Tanypodinae_01 to Tanypodinae_11; Orthocladiinae_01 to Orthocladiinae_04). How be sure that organisms belonging to the same taxon have not been included in many different (nested) morphotaxa of the faunal list, especially if difficult to identify at species level?

What could be the impact of this series of methodological decisions on the quantification of pond assemblage diversity defined on taxonomic criteria (e.g. richness)?

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.

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.

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) "two-way ANOVAs without replication" simultaneously testing both effects (but three numbers of degrees of freedom should have been provided by ANOVA).

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.

Lines 250-255 and 265-276: Using both (i) a Multi Factorial Analysis (MFA) to explore the relationship between the "environmental parameters" (in fact, land-use descriptors only) and macroinvertebrate-based diversity metrics and (ii) a Redundancy Analysis (RDA) to identify environmental parameters (in this case, physico-chemical parameters measured in water or sediment) best explaining variation in taxonomic structure of assemblages should be briefly justified. Why not applying only one of these approaches to the whole set of environmental variables (i.e. both land use and physico-chemical descriptors)?

MFA typically deals with datasets where variables from different sources (here, taxonomic and land use) are organized in groups, highlighting a common structure of all the groups, and the specificity of each one. In your case, the three variables describing the land use (the second group of variables; i.e. "%urbanised areas", "%agricultural areas" and "%grassland and forest") do not make it possible to distinguish the surveys carried out on the same station over the 4 campaigns (since their description is certainly similar), which undoubtedly explains the particular position (aligned) of the 4 samples carried out on each pond in the first factorial plane of the MFA (fig. 4c), given the levels of correlation of the corresponding variables with the first two axes (fig. 4b) and also the particular shape of the associated confidence ellipses (completely flat).

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

For MFA, interesting information can be found in Abdi et al. (2013).

Cited reference:

Abdi H., Williams L.J., Valentin D. 2013. Multiple factor analysis: principal component analysis for multitable and multiblock data sets. WIREs Computational Statistics, 5, 149-179. 2013. https://doi.org/10.1002/wics.1246

Results:

Line 290: More probably "morphotaxa richness" and not "species diversity".

Lines 360-363 and figure 3: How may be ecologically interpreted morphotaxa gains and losses if morphotaxa can be different instars in a given taxon?

The X-axis and Y-axis labels (i.e. "species" gains or losses) are somewhat abusive.

The "solid line" is not green but red and the "dashed line" is not red but green. As a result, it becomes difficult to interpret the figure based on its legend. Adapt the figure caption.

The authors have indicated in the figure legend: "The position of the red line below the green line in all comparisons shows that morphotaxa losses dominated the gains". Can the lower panels (C1-C2 and C3-C4 sessions) be considered as illustrations of the seasonal effect and the upper panels (C1-C3 and C2-C4 sessions), with greater differences, as illustrations of the interannual effect (including the change in sampling strategy)?

Lines 380-392 and Figure 4: See my comment above (on lines 250-255 and 265-276).

Lines 399-434 and figure 5:

Why the variable arrows on the biplots (i.e. the standardized canonical coefficients?) don't have exactly the same length on the panels a (morphotaxa) and b (ponds) – and similarly on the panels c and d – even if these panels correspond to the same analysis?

For readability, it would have been more judicious to use the same scale for the abscissas on the one hand, for the ordinates on the other, in the two figures corresponding to the same analysis.

Discussion:

Lines 444-448: It is difficult to know the relative contribution of the effect of the change in sampling method and the effect of inter-annual variability (e.g. related to difference in anthropogenic pressures or weather conditions).

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).

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?

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).

Probably, the metrics used to describe diversity, only based on morphological/taxonomic criteria, were not optimal to highlight significant relations between land use and morphotaxa diversity variations. Why did the authors base the analysis solely on taxonomic criteria and not (also) on functional criteria, e.g. via the analysis of biological traits in macrobenthic communities? When only "common" taxa are captured, there may be more interesting information to draw from examining variations in the selection of biological adaptations within invertebrate communities.

Minor points:

- Line 106: "different" or "differed"?
- Lines 132-133: Is there a form of gradient from A to L or have these codes been allocated randomly?
- Line 150: "net pond" or "pond-net"?
- Line 177: Change "Poisson" in "Poisson, 1957".
- Line 179: "Taxonomic rank" or "taxonomic level"?
- Supplementary material; Table S1 (and lines 215-220 in the main text): This table is intended to provide an exhaustive list of pesticides and pharmaceuticals measured in water and sediments, in which only compounds in bold have been included in the analysis … but it seems that none of the 47 compounds listed in the last column are in bold type. Please, harmonise the table with its legend and the last paragraph of the sub-section "Determination of water and sediment quality parameters, including trace elements and organic pollutants", which exhaustively provides the micropollutants included in the analyses.
- Line 482: Change "Antipodarum" in "antipodarum".
- Line 519: Change "Proasselus sp." in "Proasellus sp.".
- Lines 599-722: All the references are not provided in a homogeneous format. Some of them need marginal edits:
- Line 654: Change "Forest Ecosystems 2019; 6" in "Forest Ecosystems 2019; 6: 7".
- Line 660: Change "Science of the Total Environment 2020; 740" in "Science of the Total Environment 2020; 740: 140029".
- Line 663: Change "Environ Sci Pollut Res 2020" in "Environ Sci Pollut Res 2020; 28, 59256–59267".
- Line 667: Change "Ecosphere 2019; 10" in Ecosphere 2019; 10: e02810".
- Line 674: Change "Science of the Total Environment 2021; 773: 12" in "Science of the Total Environment 2021; 773: 145467".
- Lines 690: Change "Sun ZH" in "Sun Z".
- Line 710: Change "Environmental Monitoring and Assessment 2021; 193: 11" in "Environmental Monitoring and Assessment 2021; 193: 694".

Reviewed by **Aurélie GOUTTE**, 18 January 2024

The manuscript entitled "Do macroinvertebrate abundance and community structure depend on the quality of ponds located in peri-urban areas?" deals with spatial (12 sites) and temporal (spring and fall in two consecutive years) diversity of macroinvertebrate communities and the effects of physicochemical parameters and contaminants (trace elements, pharmaceuticals, pesticides and polycyclic aromatic hydrocarbons) on abundance and community structure.

This is an interesting and original study, the manuscript is well-written and the scientific approach is clearly presented.

I only have a few comment and questions:

- I am more familiar with techniques used in lotic systems than in ponds for macroinvertebrates inventories. I am a bit surprised to see no reference in the methods (lines 159-169). Do you think that the method you have developed here can be extended at a larger scale and used as a reference? Can you include a critical opinion, recommendations and improvements in the discussion?
- For I2M2, some species characteristics and functional traits are considered, such as the relative abundance of polyvoltine taxa or ovoviviparous taxa, which can be pertinent indicators of environmental perturbations. Do you think that these traits can be helpful in ponds and that they could be considered in your study, in addition to Shannon index, evenness, etc?
- Lines 230-231 : "We tested the effect of individual ponds and field campaign on these parameters with an analysis of variance followed by a pairwise comparison with Tukey's HSD test" Since each pond was sampled 4 times, mixed models with repeated measures can be more appropriate.
- Several environmental parameters were measured and included tested. I wonder if the number of ponds (N=12) was sufficient for testing all these parameters.
- I have noticed a few grammatical errors, for instance line 106-107 "the water contamination profiles of these ponds different depending on their location"

Reviewed by anonymous reviewer 2, 04 February 2024

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