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Do macroinvertebrate abundance and community structure depend on the quality of ponds located in peri-urban areas?

Florence D. Hulot[1*], Christophe Hanot[1], Sylvie Nélieu[2], Isabelle Lamy[2], Sara Karolak[1], Ghislaine Delarue[2], Emmanuelle Baudry[1]

[1] Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systematique Evolution, 91190 Gif-sur-Yvette, France
[2] Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, 91120 Palaiseau, France

*Corresponding author
Florence.hulot@universite-paris-sacly.fr

ABSTRACT

Contamination is one of the major threats to freshwater biodiversity. Compared to other aquatic ecosystems, peri-urban ponds are unique because they are embedded in human-dominated areas. However, it is poorly understood how different land uses such as urban or agricultural contribute multiple pollutants to ponds and thus affect pond biodiversity. In this work, 12 ponds located in a peri-urban area (Ile-de-France region, France) were monitored for 2 consecutive years in spring and fall. We surveyed macroinvertebrates and measured the physicochemical parameters and contaminants of different classes (trace elements, pharmaceuticals, pesticides and polycyclic aromatic hydrocarbons) in both water and sediment. The objective was twofold: (1) to explore local and regional macroinvertebrate spatiotemporal diversity and (2) to understand the effects of contaminants on community structure. We observed 236 macroinvertebrate morphotaxa, none of which were rare or sensitive to pollutants. Morphotaxa richness showed small differences between ponds but no difference between there was no effect of field campaign. There was no effect of ponds and field campaign on morphotaxa diversity and equitability. We did not observe a relationship between land use around the pond (agricultural, urban, or semi-natural) and diversity indices with the exception of the proportion of agricultural land in the vicinity of the pond on equitability. Regional beta diversity (between ponds) showed that differences in morphotaxa composition reflected species replacement more than differences in species richness; these were primarily due to the high abundance of pollutant-tolerant species in some of the ponds. The effects of environmental parameters on community structure were studied using partial redundancy analysis based on the presence-absence

of morphotaxa, showing that community assemblages are shaped by sediment levels of pharmaceuticals, water conductivity and ammonium concentration. In conclusion, ponds in peri-urban areas are exposed to various human activities, with our results suggesting that this exposure leads to chronic and diverse contaminations that affect morphotaxa communities.

Keywords: beta diversity, land use, pollutant-tolerant morphotaxa, trace elements, PAH, pharmaceuticals, herbicides, insecticides, fungicides

1. Introduction

Freshwater ecosystems, including lakes, reservoirs and rivers, host around 9.5% of all described species despite representing approximately 2.3% of the world's surface area and only 0.01% of its water (Reid et al. 2019). They therefore deserve special attention in terms of their protection and conservation. In a recent review, Reid et al. (2019) listed 12 emerging or intensifying threats to freshwater biodiversity. Among the different freshwater ecosystems, ponds are particularly vulnerable because of their small size (Biggs et al. 2017), although they are understudied probably on account of their presumed insignificance (Biggs et al. 2017; Cereghino et al. 2008). Several studies showed that small water bodies such as ponds, ditches and streams host a higher biodiversity than large water bodies (Biggs et al. 2017). In particular, because of their isolation or small size, they may have low local alpha diversity. However, a high dissimilarity between these aquatic systems may lead to high regional beta diversity (Clarke et al. 2008; Davies et al. 2008; Williams et al. 2004). The small habitat size of ponds may also support rare and scarce species. Scheffer et al. (2006) showed that shallow lakes and ponds support a higher richness of aquatic birds, plants, amphibians and invertebrates than large water bodies. This higher richness is accompanied by a low diversity of fish, if not their complete absence (Scheffer et al. 2006).

Peri-urban areas are characterised by complex landscapes with both agricultural and urban covers and a mixture of different uses and users (Poggi et al. 2021; Zoomers et al. 2017). They are typical zones of continuous transformation (Zoomers et al. 2017) or "restless landscape" (Friedmann 2016). However, ponds and wetlands in peri-urban environments are understudied (Wanek et al. 2021). Although urban ponds are less diverse than rural ponds, they may host threatened species, thus advocating for good management practices (Oertli

and Parris 2019). Likewise, motorway stormwater retention ponds can play a significant role in macroinvertebrate diversity at the regional level (Le Viol et al. 2009; Meland et al. 2020).

Contamination by pollutants is one of the 12 threats to freshwater biodiversity identified by Reid et al. (2019). Ponds are subjected to multiple contaminations depending on their environment, whether in an agricultural, urban or highway setting. These stressors have been studied both independently and in combination and include, among others, pesticides (Trigal et al. 2007), major ions, nutrients and wastewater-associated micropollutants (Berger et al. 2018), polycyclic aromatic hydrocarbons (PAH) (Uher et al. 2016), pharmaceuticals and heavy metals (Andreu et al. 2016), metals, ions and PAH in combination (Sun et al. 2018). The distinctive feature of peri-urban ponds is that they are embedded in a human-dominated matrix with different activities, which can be the source of multiple stressors. In peri-urban areas, although ponds are not located far from each other, they might be exposed to different dominant stressors or combine different categories of pollutants because of their small catchment area (Biggs et al. 2017; Cereghino et al. 2008). In addition, contaminants in ponds may change with time if the surrounding landscape is submitted to changes such as urbanisation or temporary construction sites, or if it is exposed to new materials such as nanomaterials or personal care product additives. Ponds may accumulate these contaminants, particularly in their sediments and, consequently, freshwater life is affected by changing contaminant cocktails.

Aquatic macroinvertebrates encompass a rich and diversified set of taxa that are universally found in freshwater ecosystems. They exhibit a wide range of sensitivity to environmental stressors and, as a consequence, their local diversity and abundance are commonly used as

indicators of perturbations (Sumudumali and Jayawardana 2021; Tachet et al. 2010). Aquatic macroinvertebrates are mostly sedentary, at least during their larvae stage. They inhabit different habitats and their life cycle, for the majority of macroinvertebrates, is annual (Tachet et al. 2010). For these reasons, these organisms are good indicators of pollution, as the recolonisation of perturbed areas takes time. Macroinvertebrate indices of water quality are based on the presence-absence or abundances of macroinvertebrates. However, in addition to the local diversity known as alpha diversity, spatial and temporal diversity give information respectively about the spatial structuration and temporal changes of communities (Legendre and Condit 2019). Thus, their spatial and temporal beta diversity might prove useful to assess the impacts of stressors in changing peri-urban areas.

Here we report the results of a study in which we monitored 12 ponds in a peri-urban area located in the Ile-de-France region (France). The chosen ponds were characterised by different proportions of agricultural, urbanised, grassland and forest surfaces in a 100-m radius buffer. We therefore aimed to link land use, contaminant concentrations in water and sediment as well as macroinvertebrate distribution in ponds. Our objective was to understand whether water and sediment pollutants and land-use characteristics are constraints to macroinvertebrate distribution in ponds. To do so, we sampled macroinvertebrates and measured pond quality parameters, including various urban and agricultural contaminants, to quantify the main pesticides, pharmaceuticals, PAH and trace elements (TE) in water and sediments. Depending on their chemical properties, contaminants may accumulate either in water or in sediment. We therefore monitored both compartments. Nélieu et al. (2020) showed that the water contamination profiles of these ponds differed depending on their location, and that the agricultural landscape explained these differences more than urban land

uses. In some of the ponds, the environmental risk exceeded the thresholds of risk quotient mainly due to pesticides (Nélieu et al. 2020). Here, we analysed the macroinvertebrate communities and hypothesised that (1) local macroinvertebrate diversity is higher in ponds located in environments dominated by grasslands and forests than in those dominated by agriculture and urban areas; (2) ponds hosting rare and pollutant-sensitive macroinvertebrate morphotaxa highly contribute to regional diversity; and (3) water and sediment contaminants influence morphotaxa distribution in ponds.

2. Material and methods

2.1. Study area

The selected study area is the Saclay Plateau (N: 48° 43' 59.99" E: 2° 10' 0.01), located in the junction zone between the Parisian agglomeration and its large surrounding plains. Until recently, the territory had a mainly agricultural vocation, although the ongoing development of a scientific and technological pole in the area has considerably increased the urban hold on the territory. At the same time, the Natural, Agricultural and Forest Protection Zone of the Saclay Plateau extending over more than 4,000 ha was created in 2010, thus perpetuating the agricultural use of land on the plateau. The Saclay Plateau thus presents major challenges in terms of the coexistence of urban and agricultural areas and biodiversity in a context of growing urbanisation. Within this plateau, we selected 12 ponds with surface areas ranging from 64 to 828 m^2 (mean ± SD: 540 ± 320 m^2; median: 566m^2) and with different potential exposures to agricultural and urban activities. The proportion of agricultural and urbanised surface around each pond was calculated in a 100 m radius buffer. 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 zone. To calculate the different types of surface, we used the map of mainland France land cover produced by the Theia land data services using Sentinel 2 and Landsat 8 data (Theia 2017). The proportion of urbanised land was calculated by adding together three Theia categories: dense built-up areas, diffuse built-up areas and industrial and commercial areas. The proportion of agricultural land was calculated by adding two Theia categories: winter crops and summer crops. The remaining areas consist of forest and grassland. In the following, we refer arbitrarily to ponds A to L.

2.2 Sampling methods for invertebrates and for water and sediment

The sampling of invertebrates as well as water and sediment was systematically performed at the same points in the 12 ponds. To ensure that water and sediment sampling and invertebrate collection did not interfere with each other, they were carried out with a time lag of about 1 week.

A preliminary analysis was conducted to determine the most appropriate timing for sampling the invertebrates in the ponds. Several ponds were sampled every month from May to October 2015 inclusive, which are the periods during which most species are active in our geographical area. Eight of these ponds were included among the 12 ponds selected in our study. Non-metric multi-dimensional scaling (NMDS) analysis of the collected taxa showed that sampling in June and September covered almost all the diversity of the macroinvertebrate species present in the ponds (Hanot, unpublished results). Sampling was thus performed in June and September during 2016 and 2017 in the 12 ponds selected for the analysis. In the following, we refer to the field campaigns as C1 (2016 June), C2 (2016 September), C3 (2017 June) and C4 (2017 September).

Macroinvertebrate sampling was carried out on two different main habitats on either side of each pond to best represent the biodiversity of the ponds in a standardised manner. Sampling was made using a pond-net with a 77 cm fiberglass handle, a trapezoid frame measuring 39 x 14 cm x 32 cm, a 1 x 1 mm mesh and a pocket depth of 40 cm. Samples were collected by making an infinity symbol by hand eight times (i.e., a figure eight on its side), ending with a fast move from the bottom to the top in the axis point of the symbol, to collect organisms trapped in the vortex created by the sequence of movements. During this sequence, the frame

of the net pond was a few centimetres above the substratum, which allowed us to lift and collect the benthos thanks to the upward current created by the “infinity” movement. To sample each pond in the same way, the same operator made the movements at an arm’s distance from the bank. In a small number of cases, the water level in the ponds was too low to sample from the selected habitat. In this case, no collection was made at this point or at any other to ensure that the samples were collected in the same location during the entire study period.

During the collections in 2016, the content of the net pocket was quickly placed in a white basin measuring 80 x 40 x 10 cm. All specimens were sorted by eye and fixed in absolute ethanol 99% (Fisher Chemical, CAS 64-17-5) using entomological forceps. Collection stopped when 5 min had elapsed without seeing any moving specimen. Thereafter, all samples were kept at 7°C until the identification stage. During the 2017 campaigns, to speed up the sample collection, water was squeezed from the net pocket as gently as possible to avoid destroying the specimens, and the result was then fixed in absolute ethanol 99% in suitable containers and kept at 7°C. Specimens were sorted using a Motic SMZ 171 binocular microscope and entomological forceps, fixed again in absolute ethanol 99% and then kept at 7°C. Our sampling protocol resulted in 88 invertebrate samples: 12 ponds x 2 points x 2 years x 2 seasons, minus 8 cases (4 ponds x 2 points) when the collection was not possible due to a low water level. Invertebrate identifications (see next Section) were carried out separately for each sample.

In 2016 and 2017, in each of the 12 ponds, water and sediment samples were collected in spring and autumn from the same two points selected for invertebrate sampling. The water

samples were taken approximately 1 m from the edge of the ponds using a stainless steel beaker with an extendable handle. Sediment sampling was performed with the same device used to collect the water samples.

2.3 Identification of invertebrates

Different books (Bameul 1985; Guignot 1947; Hansen 1987; Holmen 1997; Jansson 1986; Olmi 1976; Poisson 1957 ; Tachet et al. 2010) and websites (http://www.perla.developpement-durable.gouv.fr/, http://coleonet.de) were used as a reference to perform the morphological identification of all specimens under a Motic SMZ 171 stereo microscope. Identifications were made at the lowest possible taxonomic level. Specimens 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). Because of these different levels of determination, we hereafter refer to specimens as “morphotaxa”, which are defined as taxa that share the same morphological characteristics. When it was not possible to link the different stages (larva, nymph, adult) to the same species, they were assigned to different morphotaxa. Some specimens of each taxon were kept in tubes of 2 mL, 5 mL or 40 mL according to their size, in absolute ethanol 99%, to be used as a reference. This made it possible to constitute a reference base for the invertebrates in the ponds by linking the reference specimens with their morphotaxon names. Each specimen of each sample was then identified using books, websites and the reference base. All specimens were counted by morphotaxa for each sample.

2.4 Determination of water and sediment quality parameters, including trace elements and organic pollutants

The choice of contaminants assessed here was based on the local activities: cereals, maize, rapeseed, sunflower, orchard and vegetable crops for pesticides; nearby roads for TE and PAH; and the presence of humans, farms, and domestic pets for pharmaceuticals. Samples were used to determine the main physicochemical parameters, including major and TE as well as organic contaminants (PAH, pesticides and pharmaceuticals) as described in Nélieu et al. (2020).

For the water samples, the following measurements were taken directly on site with probes: pH, conductivity, temperature, dissolved oxygen (DO) and turbidity. Other data were obtained rapidly in the laboratory (mainly within one day of sampling) using standardised methods: dissolved organic carbon (DOC) by thermic oxidation and IR analysis of carbon dioxide, chemical oxygen demand (COD, norm NF EN ISO 15705), suspended solids (SS, norm NF EN 872), nitrates (NO_3^-, norm NF EN ISO 10304-1), nitrites (NO_2^-, NF EN ISO 26777), total nitrogen (TN) from the addition of Kjeldahl nitrogen (Kjeldahl method, norms NF EN 25663 and NF EN ISO 11732) with nitrates and nitrites, total phosphorus (P, norm NF EN ISO 15681-2 and NF EN ISO 6878.), anions (norm NF EN ISO 10304-1) and cations (norm NF EN ISO 14911), as well as major and TE (norm NF EN ISO 17294-2: Al, As, B, Be, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sn, U and Zn), 15 PAH, pesticides (25 herbicides, 1 safener, 7 fungicides and 2 insecticides) and 12 pharmaceuticals compatible with the multi-residue method applied after sample conservation at -20°C (see the exhaustive list in Table S1). Details on the methods used for this determination can be found in Nélieu et al. (2020).

Sediment samples were used to determine the contents in organic carbon (Corg), total nitrogen (N) and thus C/N ratio (norms ISO 10694 and ISO 13878) as well as total major and TE (after HF mineralisation and then ICP-AES or ICP-MS analysis according to the norm NF X 31-147/NF ISO 22036 - 17294-2), for the following: Cr, Cu, Ni, Zn, Co, Pb, Cd, Tl, Mo, Al, Ca, Fe, K, Mg, Mn, Na, P (P_2O_5), Bi, In, Sb and Sn. All measurements were made in the Laboratory of Soil Analysis of INRAe (Arras, France). The polycyclic aromatic hydrocarbons were analysed according to the European standard NF EN 16181 (2018) by pressurised liquid extraction and HPLC-fluorescence quantification. The same pesticides, metabolites and pharmaceuticals selected for analysis in water (Nélieu et al., 2020) were also monitored in sediments (see Appendix 1 for the analysis methods used for sediments).

Not all the measured pesticides and pharmaceuticals were detected in water and sediment (no detection or values below the quantification limits). Therefore, our study is based on fifteen herbicides (Atrazine, Atrazine-desethyl, Simazine, Terbuthylazine, Terbuthylazine-desethyl, Clomazone, Diflufenican, Napropamid, Acetochlore, Alachlore, Dimethachlore, Metolachlore, Chlorsulfuron, Metsulfuron-methyl and Nicosulfuron), seven fungicides (Boscalid, Dimoxystrobine, Epoxiconazole, Hexaconazole, Metconazole, Picoxystrobine, Tebuconazole), two insecticides (Imidacloprid and Pyrimicarb) and one pharmaceutical (Carbamazepine).

All the data were added to the In.Do.Res repository (https://doi.org/10.48579/PRO/TX0PU9,).

2.5 Statistical analysis

Prior to the data analysis, the invertebrate samples collected from both points in each pond were pooled. This allowed us to produce a contingency table containing the number of each morphotaxon for each pond and each sampling date. For the water quality parameters, TE and organic pollutants, we computed the mean of the two sample point values. 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. All statistical analyses were performed with R 3.6.1 (Team 2020).

We performed analyses based on morphotaxa abundance and presence-absence. However, due to differences in specimen determination levels, the analyses may be biased and should therefore be taken with caution. To explore invertebrate community diversity, we computed the morphotaxa richness (alpha diversity), Shannon diversity index and Pielou evenness for each pond and each field campaign with the specnumber function in vegan 2.5-6 (Oksanen et al. 2019). We tested the effect of individual ponds and field campaign on these parameters with an analysis of variance with additive effects of pond and field session followed by a pairwise comparison with Tukey's HSD test.

To study the dissimilarities between invertebrate communities, we computed the total beta diversity within each field campaign across all ponds using the beta.multi function of the betapart package 1.5.2 (Baselga et al. 2020). This function partitions the total beta diversity into two additive components, turnover and nestedness, which reflect species replacement and species richness difference, respectively (Baselga et al. 2020).

We calculated the contribution of each pond to beta diversity, that is, the local contribution to beta diversity (hereafter LCBD) following Legendre and Cáceres (2013) using the beta.div function of the adespatial package 0.3-8 (Dray et al. 2019). As pond dissimilarity may be different if computed with the abundance or presence-absence (PA) of morphotaxa, we calculated both using the Hellinger and Jaccard dissimilarity coefficients, respectively. The LCBD values, which represent the uniqueness of a pond in terms of taxa composition, were tested for significance with the null hypothesis of a random distribution of species among ponds within a sampling campaign (Legendre and De Cáceres 2013). We also computed the species contributions to beta diversity (hereafter SCBD) to identify the morphotaxa that contribute the most to beta diversity. This last index is calculated only with morphotaxa abundance. We explored the temporal effects (year and season) on morphotaxa assemblages but as the results are not robust, we present them in Appendix 2.

To explore the relationship between the parameters describing diversity and land use, we tested the effects of the proportion of urban, forested and agricultural areas on morphotaxa richness, diversity, equitability and LCBD with a linear mixed effect model with the field campaign as a random effect. We used the lme4 package, v.1.1-23 (Capps et al. 2015).

Finally, we explored the relationship between the environmental parameters and the macroinvertebrate assemblages to identify the parameters that best explain the community structures. The concentrations of water TE, PAH and pharmaceuticals were very low, being at the detection limit; for this reason, we did not include them in the analyses. Prior to the analyses, to reduce the high number of TE in the sediments, we performed principal

component analysis (PCA) in all ponds for the four field campaigns. The first two axes of the PCA explain 68.67% of the variability (Figure S1). The ponds are arranged on the first axis (TE1) from low to high TE concentrations. The second axis (TE2) discriminates ponds with high concentrations of TE (Sb, Cd) from those with high concentrations of major elements (Na, Mg, Fe). We collected the coordinates of each pond-field campaign combination for the first two axes of the PCA and then used them in the following statistical analyses as summaries of each contaminant group effects on ponds. We summed the concentration of the different PAH and used the total PAH concentration in the following analyses. We used redundancy analysis (RDA) (Borcard et al. 2011) and ran two analyses, one with the Hellinger-transformed abundances of morphotaxa and another with the PA of morphotaxa. In the RDA, the response matrix is the abundance or PA of morphotaxa in all ponds and the four field campaigns, whereas the explanatory matrix is the environmental parameters, including the contaminants for the same pond-field campaign combination. We used the following parameters in the RDA: (1) for water: conductivity, suspended matter, COD, DOC, concentrations of TN and phosphorus, orthophosphate, ammonium, organic carbon, herbicides and insecticides; and (2) for sediments: herbicides, fungicides, insecticides, pharmaceuticals, PAH and the first two PCA axes performed with TE.

We used the rda function of the vegan package v2.5-6 (Oksanen et al. 2019). We tested the significance of the two RDA results by the permutation of the overall analysis and each axis. The two RDA were significant with a threshold level of 5%. We tested for linear dependencies among the explanatory variables and computed the variance inflation factors (VIF) of the variables with the vif.cca function. We computed the adjusted R^2 with the RsquareAdj function. The VIF was very high for several explanatory variables and so to reduce the

correlations between them, we computed a forward selection of the explanatory variables using the forward.sel function. The method produces parsimonious models, which we have tested by permutation and VIF.

3. Results

3.1 Morphotaxa richness, Shannon diversity index and evenness

In total, we identified 236 morphotaxa, which represent a total of 22 orders and 54 families including 13 orders and 42 families for arthropods and 8 orders and 37 families for insects (some morphotaxa could not be assigned to an order or family). The Baetidae *C. dipterum* was ubiquitous during campaigns C1, C2 and C3 but totally absent in C4. Morphotaxa richness ranged from 7 to 49 with a median value of 25. Statistical analyses show a weak effect of pond on morphotaxa richness (Figure 1 and Table S2). Morphotaxa richness was significantly higher in pond J than in ponds E ($p=0.023$) and I ($p=0.035$). Morphotaxa richness changed between field campaigns ($p\leq 0.001$, Table S1), being significantly higher in C1 and C2 than in C4 (respectively $p=0.004$ and $p=0.008$). The Shannon index and evenness ranged from 1.07 to 2.9 and from 0.33 to 0.82, respectively. The field campaigns and ponds had no effect on the Shannon index. Only the field campaign had an effect on evenness ($p=0.02$) with a higher evenness in C4 than in C1 ($p=0.02$).

3.2 Beta diversity: Spatial dissimilarities between ponds

The total beta diversity based on morphotaxa PA was very similar for each field campaign with values between 0.92 and 0.93 (Table S3; for comparison, results for analyses based on morphotaxa abundances are in Table S2). The turnover, which reflects the level of species replacement between ponds as opposed to species loss, represents between 88% and 90% of this total beta diversity. The LCBD of each pond based on morphotaxa PA varied across the field campaigns (Figure 2, Table S3). Pond E has a significant contribution to regional biodiversity in C1 and C2. Pond I makes a significant to highly significant contribution during the four field sessions and ponds J and L in C4 and C3, respectively.

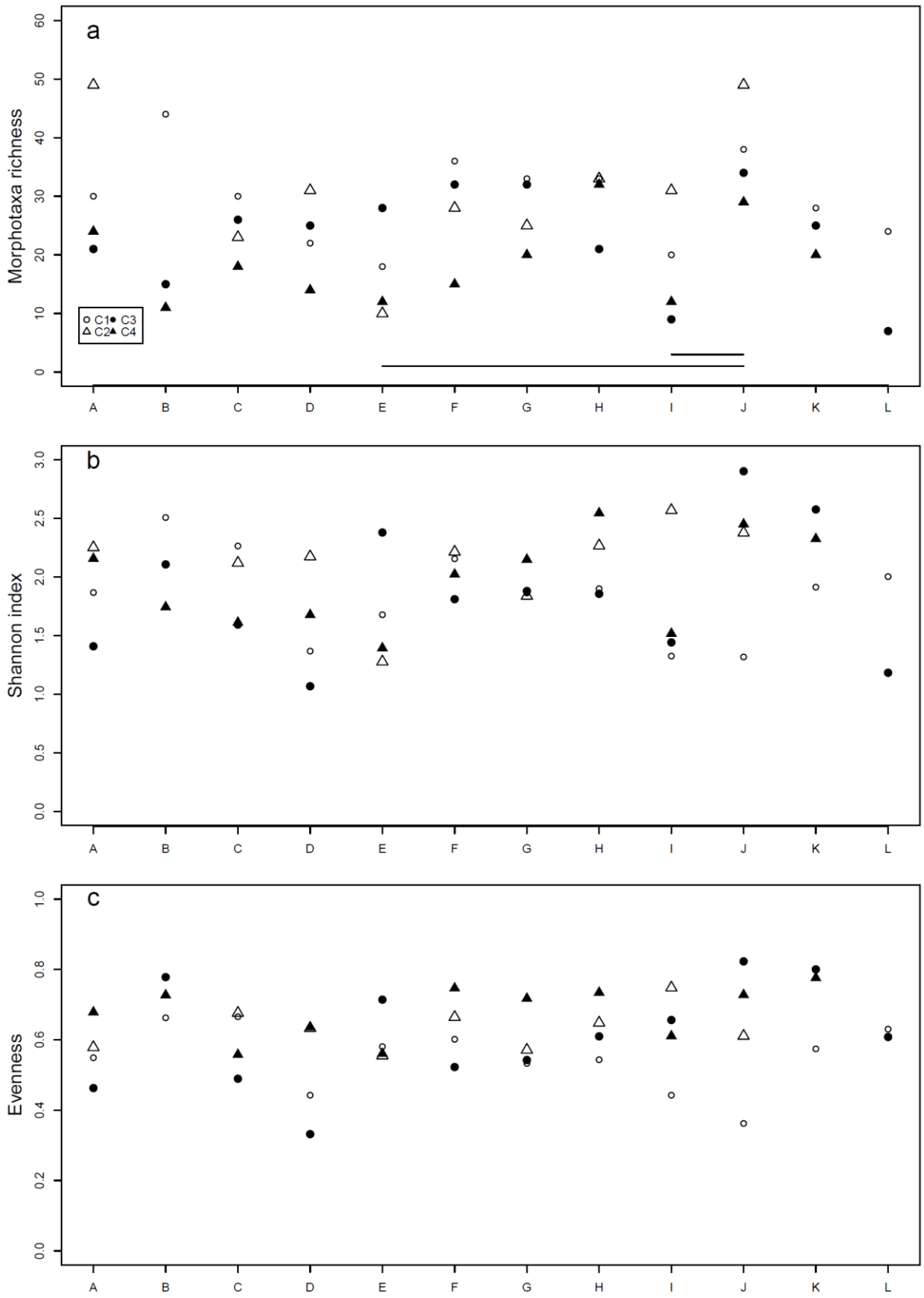

Figure 1. Morphotaxa richness (a), Shannon index (b) and evenness (c) in the 12 ponds for the four field sessions. The legend for the four field sessions is given in panel (a). The horizontal segments in panel (a) link the ponds significantly different.

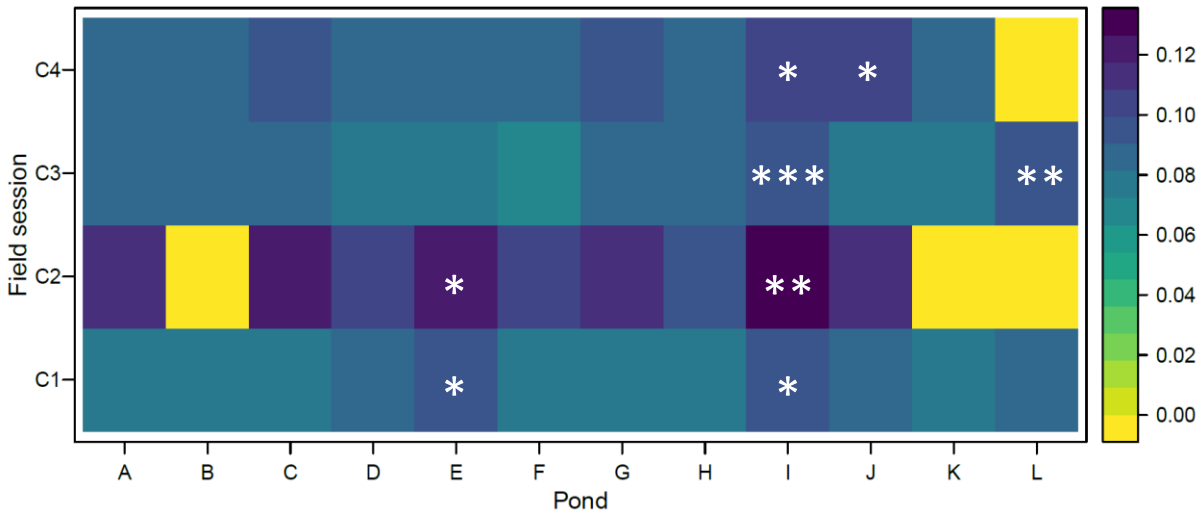

Figure 2. Local contribution to beta diversity (LCBD) of each pond for the four field sessions. The LCBD is computed with the presence-absence of morphotaxa. The LCBD are computed independently for each field session. The symbols show the significant contributions with: ***: $p \leq 0.001$; **: $0.001<p \leq 0.01$; *: $0.01 < p \leq 0.05$.

To shed light on the LCBD results, we briefly present the Species (here morphotaxa) Contribution to Local Biodiversity (SCBD) based on morphotaxa abundances. The analysis reveals that a relatively low number of morphotaxa explains most of the dissimilarities among ponds. The 15 morphotaxa with the highest contributions are listed in Table S5 and the comprehensive list of morphotaxa SCBD is provided in Table S6. The 15 morphotaxa with the highest contributions account for 83%, 73%, 71% and 70% of the total SCBD in the C1, C2, C3 and C4 field campaigns, respectively. Some are common to all four campaigns (*Asellus* sp., two different *Chaoborus* sp., different Chironomini morphotaxa) or to three campaigns (*Cloeon dipterum*; Clitellata; *Physella acuta*; *Valvata macrostoma*).

Table 1. Results of the redundancy analyses (RA) parsimonious models

Variables	Cumulative adjusted R^2	F value	*p* value
Water conductivity	0.032	2.401	0.001
Sediment pharmaceuticals	0.057	2.111	0.001
Water ammonium concentration	0.077	1.922	0.006

3.3 Relationship between land use, environmental parameters and macroinvertebrate assemblages

The linear mixed effect model testing the effects of the different types of land use around the ponds on parameters characterising the macroinvertebrate diversity showed that equitability increased significantly with the proportion of agricultural area in pond vicinity (p=0.026). Morphotaxa richness and diversity, and LCBD were not significantly affected by land use.

To clarify the relationship between the environmental parameters for water and sediment and the macroinvertebrate assemblages, we ran redundancy analysis (RDA) with morphotaxa PA. The initial model with all the environmental parameters was significant (p = 0.009). As some of these parameters had strong collinearities (as shown by the high VIF values), a forward model selection procedure was used to obtain a more parsimonious model. The final model describing the morphotaxa PA contained three explanatory variables: conductivity, pharmaceutical concentration in sediments and ammonium concentration in water (Table 1). The parsimonious model is highly significant (Df = 3, 40, F-value = 1.740, p=0.002) with no strong collinearity between the variables (all VIF are around 1) and is reduced to one significant canonical axis (Figure 3). Pond C is associated with high concentration of pharmaceuticals in the sediment; pond G is also associated with high concentration of pharmaceuticals in the sediment in addition to high water conductivity.

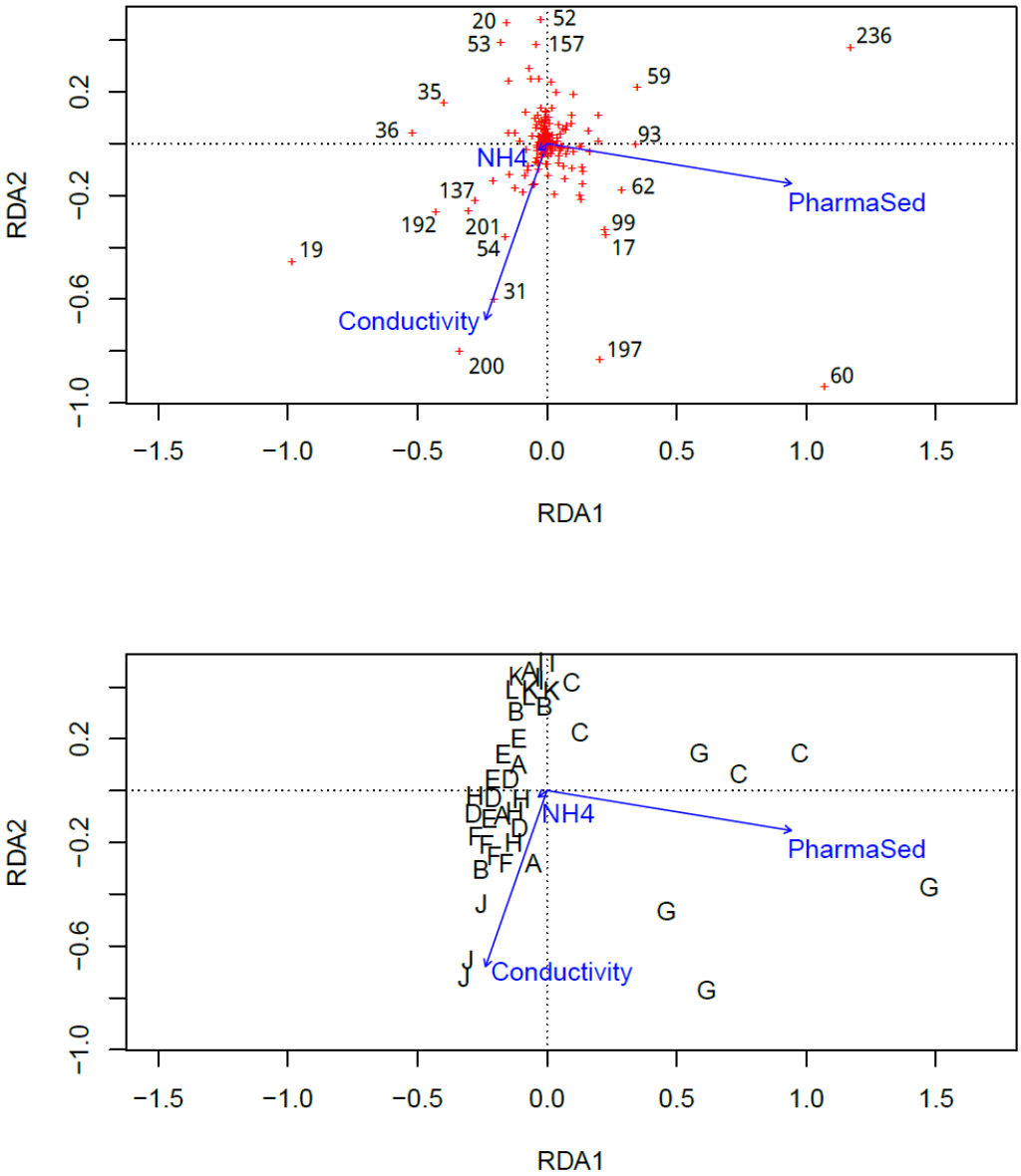

Figure 3. Parsimonious redundancy analysis (RDA) based on morphotaxa presence-absence. The biplots show the variables (blue arrows) with either the morphotaxa (numbers, panel a) or the ponds (letters, panel b). The number of morphotaxa is shown in brackets: Anophelinae 01 (17); *Asellus* sp. (19); Baetidae 02 (20); Ceratopogoninae 02 (31); *Chaoborus* sp. 01 (35); *Chaoborus* sp. 02 (36); Chironomini 04 (52); *Chironomus* sp. 01 (53); *Chironomus* sp. 02 (54); Clitellata 01 (59); *Cloeon dipterum* (60); *Coenagrion* sp.01 (62); *Dugesia* sp. 2 (93); *Erythromma viridulum* (99); Hesperocorixa 03 (137); *Hygrotus inaequalis* (157); *Physella acuta* (192); *Plea minutissima* (197); *Potamopyrgus antipodarum* (200); *Proasellus* sp. (201); and *Valvata macrostoma* (236). Conductivity: water conductivity; PharmaSed: concentration of pharmaceuticals in sediment; NH4: water ammonium concentration.

Pond J is associated with high water conductivity. Ponds, A, B, D, E, F, H, I, K and L are less affected by environmental parameters although ponds K and L seem associated with low

water conductivity. A few morphotaxa stand out and are associated with some environmental variables (morphotaxa scores in the RDA are in Table S7). *Valvata macrostoma* is associated with high pharmaceutical concentrations in sediment; *Potamopyrgus antipodarum* is associated to high water conductivity; *Cloeon dipterum* and *Plea minutissima* are associated to high values both variables. Baetidae 02, *Chironomus* sp. 01, *Chironomus* sp. 02 and *Hygrotus inaequalis* are associated with ponds in which these environmental parameters have low values.

4. Discussion

4.1 Morphotaxa distribution in the ponds and effects of land use

We changed our experimental design for macroinvertebrate sampling between the 2 years of the study. The results show a greater morphotaxa richness in the first year compared with autumn in the second year. The morphotaxa diversity was not affected and the eveness slightly affected by the field campaign. As a consequence, it is difficult to conclude on the effects of the protocol change, as the effects may be small or may have been buffered by an annual effect.

Our analysis is based on morphotaxa determined at different taxonomic levels, which is open to criticism. The aim of our work is to compare the response of assemblages to the presence of pollutants and not to compare the diversity of the ponds studied with other ponds. In this sense, questions of determination level are less important, since the same precision has been maintained for all samples. Furthermore, studies on interaction networks have shown that the level of determination of specimens has little effect on network characteristics, provided that this level of determination does not fall below too high a threshold (Llopis-Belenguer et al. 2023; Renaud et al. 2020).

Among the 236 macroinvertebrate morphotaxa identified, we did not find endangered, vulnerable or even rare species. However, we observed exotic species: the molluscs *Potamopyrgus antipodarum* and *Physella acuta* and the crayfish *Procambarus clarkii* listed as an invasive alien species in the European Union (European Union 2016). Molluscs and crustaceans are the most frequent freshwater macroinvertebrate invaders (Oertli and Parris 2019; Patoka et al. 2017). The pet trade is one of the main introduction pathways, and both mollusc species can "hitchhike" on intended shipments (Patoka et al. 2017). One individual *P. clarkii* was found in pond F in C3. Pond F also hosted the two exotic molluscs, and pond J hosted *P. antipodarum*. We should stress here that pond F, though in a forested area, is located on the Paris-Saclay university campus with heavily frequented paths in close vicinity. The campus is open to the public, which may favour the dissemination of invasive species.

Overall, our results show that the ponds distributed along an urbanisation gradient are quite dissimilar, as beta diversity relies mostly on morphotaxa turnover with a comparable morphotaxa diversity. No pond stands out consistently across the four sampling campaigns in terms of the morphotaxa contribution to regional diversity except for pond I and, to a lesser extent, pond E. The LCBD indicates the uniqueness of communities either because they are rich and host typical taxa or because they are degraded with a limited number of common taxa (Legendre 2014; Legendre and De Cáceres 2013). Here, the uniqueness highlighted by the significant LCBD points to degraded ponds. For instance, pond I has a low diversity, and its most striking feature is the absence of Baetidae in C4, whereas the mean abundance in other ponds was 81.2 individuals (± 47.0 SD). Despite the restrictions described above, we have calculated the SCBD, based on morphotaxa abundances, because it supports the idea that some ponds stand out because they are degraded. The lists of morphotaxa contributing the

most to regional diversity encompass common morphotaxa, some of which are characteristic of degraded communities such as Chironomidae, *Chaoborus* sp., *C. dipterum*, and so on. For instance, in C2, pond C was characterised by high abundances of *Chaoborus* sp. 01, *C. dipterum*, *Corixa* sp. and *V. macrostoma*, and pond E by *C. dipterum*, Orthocladiinae and Tanytarsini 01. Pond L in C3 had a low diversity and was dominated by *Notonecta* sp. 01. The uniqueness of a community as shown by a high LCBD may also indicate the presence of invasive species (Legendre 2014). However, the two ponds hosting exotic species as well as an invasive species had no significant LCBD values.

We initially hypothesised that local macroinvertebrate diversity is higher in ponds located in rural areas than in those located in agricultural or urban areas. This hypothesis is supported by different studies (Blicharska et al. 2017; Johnson et al. 2013; Noble and Hassall 2015; Thornhill et al. 2017). However, our results do not support this hypothesis, as we found only an effect of the proportion of agricultural area on morphotaxa evenness. Our results likewise do not support the second hypothesis regarding rare and pollutant-sensitive morphotaxa, as we do not observe any of them. On the contrary, we found invasive and exotic species. LCBD values could help to identify these ponds (Legendre 2014), although they only identified pond J, probably because of the very high density of *P. antipodarum*.

4.2 Effects of environmental parameters on macroinvertebrate assemblages

Our results showed that among the numerous environmental parameters and pollutants measured in water and sediment, very few are critical for macroinvertebrate assemblages in ponds. The concentration of pharmaceuticals in sediment and water conductivity are the most structuring parameters of macroinvertebrate assemblages. Although these results should be

treated with caution, the analysis with morphotaxa abundances reveal other important parameters as the concentration of fungicides in sediment as well as MTE1, insecticides, organic carbon, and COD in water. Nélieu et al. (2020) highlighted the high environmental risks due to water column pesticide concentrations in several ponds. Pesticides other than insecticides do not seem to be critical factors to explain the macroinvertebrate assemblages observed here. In contrast to sediment pollution, pollutants measured in the water column provide a snapshot into water quality; sediment pollution is relatively stable over time, and the measurements are more reliable as an indicator of pollution level (Casey et al. 2007; Sun et al. 2019). Conductivity is a general indicator of the presence of many ions in the solution, which is consistent with urban pollution associated with de-icing salts and TE (Brand et al. 2010; Oertli and Parris 2019; Wu et al. 2020). Surprisingly, conductivity is not associated with TE concentrations in our study, suggesting that these two factors do not filter morphotaxa in the same way in different ponds. Conductivity is associated with forest pond J and, to a lesser extent pond G,in addition to Ceratopogoninae, *P. antipodarum* and *P. minutissima*. Though in a rural area, pond J is bordered by a road, which may explain the high conductivity.

Based on morphotaxa abundances, we found two groups of parameters, COD and TE1, in water on the one hand, and fungicide and pharmaceuticals in sediment with dissolved insecticides on the other, generally in the same ponds but at different sampling campaigns. These ponds include G, C, B and E. Ponds B, C and E are located in an agricultural area, ponds C and G are near a farm and pond G is near a medical center. The Chironomidae Orthocladiinae, the mayfly *C. dipterum*, the mollusc *V. macrostoma* and the annelid *Clitellata* sp. characterise the assemblages found in these ponds. These morphotaxa, in particular Chironomidae and annelids, are typical of aquatic systems embedded in a degraded

environment (Hill and Wood 2014; Mackintosh et al. 2015; Wood et al. 2001). When considering the PA of morphotaxa, water conductivity is associated with pond J described above.

Compared with the analysis based on abundances, ponds C and G are characterised by high concentrations of pollutants (i.e., pharmaceuticals and fungicides in the sediment). Another set of ponds is associated with high water conductivity and major ion concentrations. This set includes pond J and, to a lesser extent, ponds A, K and L, characterised by the presence of the dipterans Ceratopogoninae, *Chaoborus* sp. and *Chironomus* sp., the Heteroptera *P. minutissima* and the Crustacea *Proasellus* sp. These ponds are mostly surrounded by grasslands and forest, with pond K being the most urbanised pond with nearby dwellings. This points to diffuse pollution associated with road traffic and occasional human activities. In both analyses, the ubiquitous *C. dipterum* is distinguished by its association with high dissolved ion concentrations and, to a lesser extent, by pharmaceuticals in sediment.

Legendre (2014) recommends using PA dissimilarity coefficients when community assemblages are characterised by a high turnover and quantitative indices when the assemblages differ in terms of abundances rather than species diversity. In our study, the high turnover in macroinvertebrate assemblages favours an analysis based on PA, thus concluding the RDA with the least factors: pharmaceuticals sediment and conductivity and ammonium concentration in water. Our third hypothesis is thus partially validated, as contaminants allow us to discriminate several ponds characterised by certain morphotaxa.

4.3 Characteristics of the peri-urban environment

The absence of a clear relationship between land use and morphotaxa diversity suggests that the presence of roads, buildings, or impervious surfaces in close vicinity to the ponds is not a critical parameter to explain the observed patterns of morphotaxa diversity at the regional scale. Instead, traces of particular activities influence morphotaxa diversity. For instance, pond A is located in a forest, which explains the hunting cartridges found in and around the pond. We did not find a high level of contaminants in this pond, although its conductivity may be due to the cartridges. Situated between a forest and fields, pond L is not far from residential buildings; people walk to this pond and brush their dogs there (we found a bristle of hairs), also allowing them to swim in the water. In this pond, Nélieu et al. (2020) found high imidacloprid concentrations, which is a veterinary pharmaceutical used to treat dog fleas and ticks. Ponds B and C are both located near farms. We observed that the farmer washed his tractor and equipment in one pond, with the wastewater running off into the pond. Pond G is located near a farm and medical center where carbamazepine, a human anti-epileptic, is used. Pharmaceuticals are markers of human activities, and carbamazepine, which is resistant to biodegradation, is only used by humans (Kasprzyk-Hordern et al. 2009). These observations illustrate the multi-functionality of peri-urban areas with a mixture of different users (Friedmann 2016; Zoomers et al. 2017) at the local scale. Peri-urban ponds combine contaminants typical of rural and urban environments, that is, a runoff of excess nitrogen (and phosphorus) and an influx of heavy metals and salt from road applications (Wanek et al. 2021). To this list, we may add pesticides and pharmaceuticals.

In their review, Oertli and Parris (2019) showed the diversity of criteria used to quantify the urbanisation of a site (presence of buildings, roads, etc.). We suggest here that local and recurrent human actions may blur these categories, particularly in semi-urban areas. Though

embedded in a semi-natural matrix, peri-urban ponds are easily reached and may encounter small, chronic and various perturbations. Several authors have advocated for well-managed urban ponds to provide high-quality habitats and support greater biodiversity (Oertli and Parris 2019; Perron et al. 2021). Peri-urban areas tend to be well described, and their potential contribution to sustainable development thus becomes more evident (Wandl and Magoni 2017). As the water quality of peri-urban ponds tends to be more similar to urban ponds than rural ones (Wanek et al. 2021), efforts should be made to manage these systems, especially since they connect rural and urban systems in the blue grid. The use of the LCBD is an interesting approach to identify the ponds to restore (Legendre 2014).

5. Conclusion

Our study of macroinvertebrates and water and sediment contaminants in 12 peri-urban ponds over 2 consecutive years reveals a high morphotaxa turnover with the absence of rare and pollutant-sensitive morphotaxa. The macroinvertebrate assemblages were relatively stable, and those contributing the most to regional biodiversity are typical of degraded ponds. The pollutants best describing macroinvertebrate PA in assemblages are pharmaceuticals in sediment and conductivity and ammonium concentration in water. Although an environmental risk due to water column pesticides could be estimated, this factor is not structuring for macroinvertebrate community. Peri-urban areas are characterised by multi-functionality with a mixture of different uses and users. Ponds located in these environments are exposed to various human activities, leading to small, chronic and diverse contaminations that affect macroinvertebrate abundance and community structure.

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Data, scripts, code, and supplementary information availability

All the data and scripts are available online: https://doi.org/10.48579/PRO/TX0PU9.
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Conflict of interest disclosure
The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article. The authors declare the following non-financial conflict of interest: Isabelle Lamy is a recommender of PCI Ecotoxicology.

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Do macroinvertebrate abundance and community structure depend on the quality of ponds located in peri-urban areas?

Supplementary materials

Appendix 1. Analyses of the sediment

To perform the extraction, duplicate sediment subsamples of 5 g were placed in 50 mL polypropylene tubes (Falcon BD) with a 15 mL mixture of 6/2/2 (v:v) methanol/McIlvaine buffer pH 8/EDTA 0.2 mM being added to each tube. The tubes were shaken on an orbital shaker (10 min, 300 rpm) and sonicated for 30 min before being centrifuged for 10 min at 1,300 g and 4°C. After collecting 10 mL of supernatant, the sediment was again extracted with 10 mL of the same mixture following the same procedure. Then, 10 mL of supernatant was collected and mixed with the first extract. To perform the purification and concentration by solid-phase extraction of the samples, they were then diluted with 100 mL of Milli-Q water, adjusted to pH 7 using 0.5 M NaOH and percolated on HRXA cartridges (500 mg, Macherey-Nagel) preconditioned successively by 5 mL of methanol, acetonitrile and water. After the percolation of the diluted extracts, the cartridges were rinsed with 5 mL of water, dried under vacuum and eluted in a 6 mL mixture of 95:5 (v/v) acetonitrile/formic acid. Samples were then evaporated under a N_2 stream, dissolved in 3 mL of 8:2 (v/v) water/acetonitrile and analysed before analysis using an ultra-high-performance liquid chromatograph (Acquity UPLC, Waters)

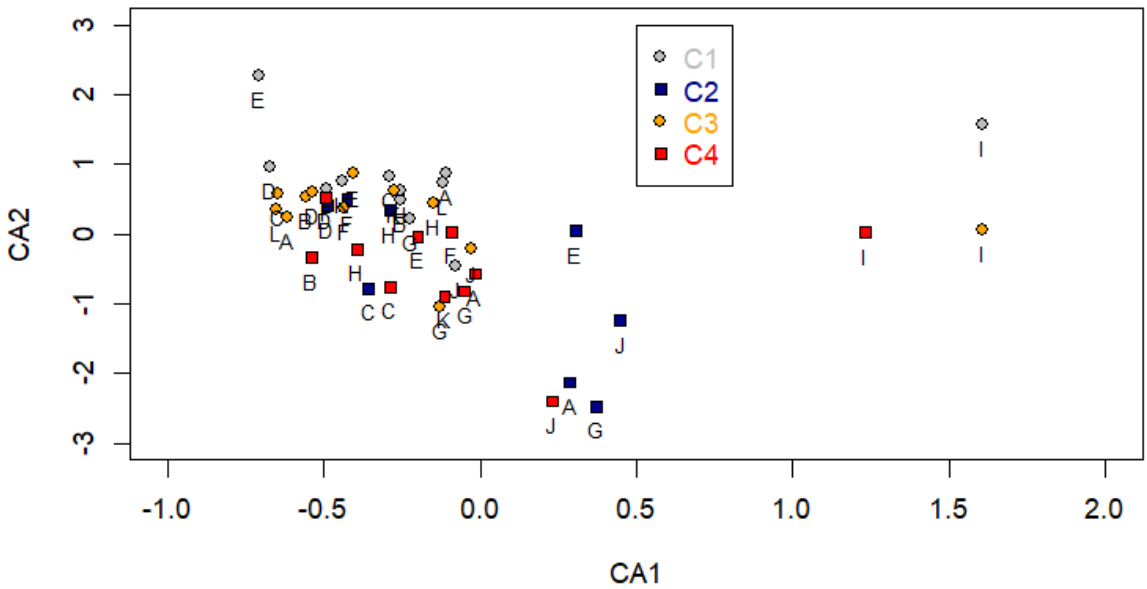
coupled through an electrospray interface to a triple quadrupole mass spectrometer (TQD, Waters). Chromatographic separation was carried out on an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size, Waters) at 30°C. The gradient profile started at 95% water with 0.1% acetic acid and linearly progressed to 55% acetonitrile with 0.1% acetic acid at 7.5 min and then 100% at 8.5 min at a flow rate of 0.3 mL/min. After a 4.5 min plateau, the initial conditions were restored and allowed to equilibrate for 2 min. The injection volume was 10 µL. The UHPLC effluent was directed to the electrospray source of the mass spectrometer, operating alternately in positive and negative modes. Capillary voltage (3.0 kV for all the analytes), cone voltage and collision-induced dissociation with argon (under a pressure of 3.4 × 10^{-3} mbar in the collision cell) were optimised individually for each compound to maximise their detection under multiple-reaction monitoring mode (MRM), as indicated in Nélieu et al. (2020). The source temperature was set at 120°C, desolvation temperature at 300°C, extractor voltage at 3 V and cone and desolvation gas flow (nitrogen) at 20 and 800 L/h, respectively (Bourdat-Deschamps et al, 2014).

The external calibration was performed with standard mixtures also analysed by UPLC-MS/MS, with the calibration curves being obtained by weighting 1/x and with residues below 20% using the QuanLynx software (Waters). The matrix effect was corrected by spiking each sample by a known amount of standard mixture in cases where the error exceeded 10%. The quantification limits are given in Table S1.

Appendix 2. Seasonal or inter-annual effects on morphotaxa abundances

We carried out a correspondence analysis (CA) with the morphotaxa presence/absence. Then we recovered the scores of the ponds on axes 1 and 2 and tested them for season * year effects with an ANOVA (Benedetti et al 2019).

The CA shows that the axes 1 and 2 explain respectively 5.6% and 4.8% of the variance. The ANOVA shows a season effect (p<0.0001) on the CA axis 2. However, as the results are not robust, we conclude that there is no season or year effect on the assemblages.



Pond scores on CA axis 1 ~Season*Year

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	1.97	1.9733	2.128	0.152

Year	1	1.16	1.1637	1.255	0.269
Season:Year	1	0.90	0.9021	0.973	0.330
Residuals	40	37.09	0.9274		

Pond scores on CA axis 2 ~Season*Year

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	11.434	11.434	17.637	**0.000145**
Year	1	0.913	0.913	1.408	0.242336
Season:Year	1	0.651	0.651	1.004	0.322414
Residuals	40	25.931	0.648		

Benedetti et al. 2019. The seasonal and inter-annual fluctuations of plankton abundance and community structure in a North Atlantic marine protected area. Frontiers in Marine Science. doi: 10.3389/fmars.2019.00214

Table S1. Exhaustive list of pesticides and pharmaceuticals measured in water and sediments. Only compounds in bold were included in the analysis. The other compounds were not detected, or their values were below the quantification limits.

Action	Family	Compound
Herbicide	Triazine and metabolites	**Atrazine**
		Atrazine-desethyl
		Simazine
		Terbuthylazine
		Terbuthylazine-desethyl
	Isoxazolidinone	**Clomazone**
	Pyridinecarboxamide	**Diflufenican**
	Alkanamide	**Napropamid**
	Chloroacetanilids	**Acetochlore**
		Alachlore
		Dimethachlore
		Metolachlore
	Sulfonylureas	**Chlorsulfuron**
		Metsulfuron-methyl
		Nicosulfuron
	Aryloxyalkanoic acid	Mecoprop
	Tricetone	Sulcotrione
		Mesotrione
	Hydroxybenzonitriles	Bromoxynil
		Ioxynil
	Phenylureas and metabolites	Chlorotoluron
		DCPMU
		Diuron
		Isoproturon
		Linuron
Safener		Dichlormid
Fungicide	Triazoles	**Epoxiconazole**

		Hexaconazole
		Metconazole
		Tebuconazole
	Carboxamides	**Boscalid**
	Strobilurins	**Picoxystrobine**
		Dimoxystrobine
Insecticide	Neonicotinoids	**Imidaclopride**
	Carbamates	**Pyrimicarbe**
Antibiotics	Quinolones	Flumequine
		Nalidix Acid
		Oxolinic Acid
	Lincosamides	Lincomycine
	Sulfonamides	Sulfadiazine
		Sulfamethazine
		Sulfamethoxazole
	Tetracycline	Doxycycline
		Tetracycline
Other pharmaceuticals	Anti-epileptic	**Carbamazepine**
	Anti-inflammatory	Ibuprofen
		Diclofenac

Table S2. Effects of the ponds and field sessions on morphotaxa richness, Shannon index and evenness.

Model	**df**	**F-value**	**p-value**
Morphotaxa richness			
Pond	11	2.55	**0.02**
Field session	3	6.41	**0.002**
Shannon index			
Pond	11	1.16	0.35
Field session	3	1.11	0.36
Evenness			
Pond	11	1.14	0.36
Field session	3	3.79	**0.02**

Table S3. Total beta diversity and its turnover and nestedness components for the four field sessions computed with the abundance or presence-absence of morphotaxa.

	Presence-absence				Abundance			
	C1	C3	C3	C4	C1	C2	C3	C4
Total beta diversity	0.92	0.92	0.92	0.93	0.91	0.89	0.92	0.87
Turnover component	0.90	0.86	0.86	0.90	0.85	0.80	0.86	0.78
Nestedness component	0.02	0.06	0.06	0.03	0.06	0.09	0.06	0.8

Table S4. Local contribution to beta diversity (LBCD) of the 12 ponds for the four field sessions and their associated probability for significance. A. LCBD based on morphotaxa presence-absence. B. LCBD based on abundances.

A.

Pond	C1		C2		C3		C4	
	LCBD	pLCBD	LCBD	pLCBD	LCBD	pLCBD	LCBD	pLCBD
A	0.078	0.827	0.112	0.421	0.085	0.375	0.089	0.608
B	0.078	0.794	-	-	0.088	0.169	0.085	0.813
C	0.080	0.748	0.118	0.088	0.083	0.533	0.096	0.141
D	0.087	0.253	0.099	0.985	0.077	0.923	0.086	0.787
E	0.097	0.011	0.120	0.050	0.080	0.767	0.084	0.857
F	0.075	0.939	0.104	0.938	0.071	0.999	0.087	0.734
G	0.081	0.645	0.115	0.219	0.089	0.112	0.091	0.506
H	0.080	0.709	0.095	1.000	0.081	0.663	0.084	0.896
I	0.095	0.027	0.127	0.005	0.098	0.001	0.103	0.021
J	0.088	0.166	0.110	0.546	0.077	0.943	0.105	0.017
K	0.074	0.961	-	-	0.076	0.966	0.087	0.714
L	0.085	0.359	-	-	0.096	0.006	-	-

B.

Pond	C1		C2		C3		C4	
	LCBD	pLCBD	LCBD	pLCBD	LCBD	pLCBD	LCBD	pLCBD
A	0.059	0.969	0.079	0.987	0.058	0.984	0.070	0.827
B	0.079	0.625	-	-	0.086	0.400	0.087	0.436
C	0.077	0.658	0.145	0.032	0.084	0.434	0.103	0.179
D	0.105	0.075	0.083	0.971	0.086	0.383	0.082	0.558
E	0.085	0.443	0.143	0.027	0.065	0.924	0.070	0.823
F	0.097	0.184	0.090	0.888	0.070	0.840	0.057	0.974
G	0.078	0.621	0.112	0.437	0.088	0.378	0.092	0.380
H	0.075	0.706	0.102	0.694	0.093	0.244	0.064	0.915
I	0.074	0.729	0.131	0.117	0.093	0.218	0.157	0.045
J	0.130	0.001	0.112	0.456	0.077	0.665	0.098	0.246
K	0.067	0.862	-	-	0.070	0.825	0.119	0.121
L	0.075	0.714	-	-	0.129	0.001	-	-

Table S5. Species contributions to beta diversity (SCBD) for the 15 most common morphotaxa and their summed contribution to the four field sessions. The SCBD calculation is based on morphotaxa abundances.

C1		C2		C3		C4	
Morphotaxa	Summed contribution	Morphotaxa	Summed contribution	Morphotaxa	Summed contribution	Morphotaxa	Summed contribution
Asellus_sp.	0.108	Tanytarsini_01	0.095	Asellus_sp.	0.149	Asellus_sp.	0.126
Chaoborus_sp._01	0.204	Asellus_sp.	0.178	Cloeon_dipterum	0.248	Chaoborus_sp._02	0.225
Orthocladiinae_03	0.298	Chironomus_sp._02	0.256	Chironomus_sp._01	0.339	Baetidae_02	0.309
Chaoborus_sp._02	0.388	Cloeon_dipterum	0.328	Notonecta_sp._01	0.389	Valvata_macrostoma	0.386
Potamopyrgus_antipodarum	0.459	Hippeutis_complanatus	0.382	Tanypodinae_02	0.437	Chaoborus_sp._01	0.451
Valvata_macrostoma	0.517	Proasellus_sp.	0.432	Chaoborus_sp._02	0.478	Chironomus_sp._01	0.503
Chironomini_04	0.574	Valvata_macrostoma	0.476	Tanypodinae_01	0.519	Physella_acuta	0.536
Chironomus_sp._01	0.625	Planaria_torva	0.521	Chironomini_04	0.551	Chironomini_04	0.566
Ceratopogoninae_02	0.674	Chaoborus_sp._01	0.565	Valvata_macrostoma	0.579	Sigara_02	0.591
Clitellata_01	0.713	Chaoborus_sp._02	0.601	Hesperocorixa_03	0.603	Clitellata_01	0.615
Physella_acuta	0.746	Orthocladiinae_04	0.634	Physella_acuta	0.627	Helobdella_sp._02	0.636
Cloeon_dipterum	0.776	Clitellata_01	0.663	Hygrotus_inaequalis	0.651	Plea_minutissima	0.656
Dugesia_sp.2	0.800	Plea_minutissima	0.690	Hyphydrus_ovatus	0.675	Hyphydrus_ovatus	0.673
Helobdella_sp._02	0.816	Chironomus_sp._01	0.716	Chaoborus_sp._01	0.694	Proasellus_sp.	0.690
Proasellus_sp.	0.831	Chironomini_04	0.737	Anophelinae_01	0.712	Sigara_03	0.707

Table S6. Morphotaxa contribution to biodiversity (SCBD) for each field session.

#	Morphotaxa	C1	C2	C3	C4
1	*Acilius* sp.	0.00036	0.00000	0.00050	0.00000
2	*Acilius sulcatus*	0.00000	0.00010	0.00025	0.00042
3	*Acroloxus lacustris*	0.00297	0.01012	0.00238	0.00261
4	*Aedes* sp.01	0.00000	0.00078	0.00000	0.00090
5	*Aeshna cyanea*	0.00025	0.00072	0.00230	0.00627
6	*Aeshna grandis*	0.00000	0.00000	0.00010	0.00000
7	*Aeshna* sp. 01	0.00017	0.00031	0.00297	0.00617
8	*Aeshna* sp. 02	0.00025	0.00000	0.00000	0.00341
9	*Aeshna* sp. 03	0.00013	0.00000	0.00036	0.00000
10	*Aeshna* sp. 04	0.00073	0.00000	0.00609	0.00000
11	*Aeshna* sp. 05	0.00008	0.00072	0.00014	0.00000
12	*Aeshna* sp. 06	0.00040	0.00000	0.00260	0.00000
13	*Aeshna* sp. 07	0.00000	0.00000	0.00516	0.00000
14	*Alboglossiphonia hyalina*	0.00000	0.00092	0.00009	0.00158
15	*Anacaena* sp. 01	0.00000	0.00036	0.00000	0.00000
16	*Anacaena* sp. 02	0.00057	0.00000	0.00000	0.00000
17	*Anophelinae* 01	0.00016	0.00349	0.01782	0.00000
18	*Armiger crista*	0.01059	0.00889	0.00304	0.00367
19	*Asellus* sp.	0.10795	0.08281	0.14862	0.12563
20	*Baetidae* 02	0.01396	0.00000	0.00000	0.08426
21	*Batracobdella* sp. 01	0.00061	0.00019	0.00145	0.00440
22	*Berosus affinis*	0.00000	0.00076	0.00000	0.00000
23	*Berosus signaticollis*	0.00000	0.00000	0.00000	0.00042
24	*Berosus* sp. 01	0.00037	0.00000	0.00000	0.00000
25	*Boreobdella* sp. 01	0.00009	0.00000	0.00000	0.00000
26	*Bythinia* sp. 01	0.00017	0.00000	0.00038	0.00141
27	*Bythinia* sp. 02	0.00000	0.00089	0.00000	0.00000
28	*Caenis* sp. 01	0.00000	0.00000	0.00038	0.00000
29	*Caenis* sp. 02	0.00000	0.00037	0.00000	0.00042
30	Ceratopogoninae 01	0.00661	0.00703	0.00046	0.00346
31	Ceratopogoninae 02	0.04913	0.00264	0.00000	0.00042
32	Ceratopogoninae 03	0.00004	0.00000	0.00000	0.00000
33	*Ceriagrion* sp 01	0.00000	0.00000	0.00000	0.01013
34	Chaoboridae 01	0.00000	0.00155	0.00000	0.00238
35	*Chaoborus* sp. 01	0.09610	0.04427	0.01925	0.06504
36	*Chaoborus* sp. 02	0.09000	0.03632	0.04116	0.09934
37	*Chaoborus* sp. 03	0.00214	0.00359	0.00185	0.00000
38	*Chaoborus* sp. 04	0.00102	0.00082	0.00041	0.00000
39	*Chaoborus* sp. 06	0.00015	0.00000	0.00000	0.00000
40	Chironomidae 01	0.00004	0.00000	0.00000	0.00000
41	Chironomidae 02	0.00000	0.00100	0.00000	0.00000
42	Chironomidae 03	0.00000	0.00037	0.00000	0.00000
43	Chironomidae 04	0.00091	0.00019	0.00000	0.00000

44	Chironomidae 05	0.00009	0.00010	0.00906	0.00000
45	Chironomidae 06	0.00020	0.00000	0.00000	0.00000
46	Chironomidae 07	0.00000	0.00036	0.00000	0.00000
47	Chironomidae 08	0.00000	0.00072	0.00000	0.00000
48	Chironomidae 11	0.00149	0.00000	0.00000	0.00000
49	Chironomini 01	0.00112	0.00051	0.00025	0.00000
50	Chironomini 02	0.00055	0.00000	0.00000	0.00000
51	Chironomini 03	0.00005	0.00000	0.00000	0.00000
52	Chironomini 04	0.05723	0.02125	0.03248	0.03042
53	*Chironomus* sp. 01	0.05097	0.02606	0.09091	0.05198
54	*Chironomus* sp. 02	0.00000	0.07784	0.00000	0.00000
55	*Chironomus* sp. 03	0.00059	0.00006	0.00038	0.00000
56	*Chironomus* sp. 04	0.00000	0.00000	0.00000	0.01219
57	*Chironomus* sp. 05	0.00105	0.00030	0.00158	0.00000
58	*Chironomus* sp. 06	0.00009	0.00000	0.00025	0.00000
59	Clitellata 01	0.03858	0.02978	0.01273	0.02339
60	*Cloeon dipterum*	0.02968	0.07220	0.09944	0.00000
61	*Coenagrion scitulum*	0.00000	0.00000	0.00000	0.00903
62	*Coenagrion* sp.01	0.00019	0.00545	0.00507	0.01045
63	*Coenagrion* sp.02	0.00000	0.00000	0.00000	0.00451
64	Coenagrionidae 01	0.00000	0.00074	0.00038	0.00000
65	Coenagrionidae 02	0.00000	0.00000	0.00139	0.00000
66	Coleoptera 02	0.00102	0.00000	0.00000	0.00000
67	*Colymbetes fuscus*	0.00000	0.00148	0.00124	0.00000
68	*Colymbetes* sp. 01	0.00000	0.00000	0.00000	0.00135
69	*Corixa panzeri*	0.00012	0.00000	0.00355	0.01230
70	*Corixa punctata*	0.00039	0.01355	0.00426	0.00570
71	*Corixa* sp.01	0.00030	0.00201	0.00151	0.00000
72	Corixinae 01	0.00063	0.00000	0.01270	0.00000
73	Corixinae 02	0.01181	0.00124	0.01261	0.00000
74	Corixinae 03	0.00000	0.00021	0.00000	0.00000
75	Crambidae	0.00000	0.00648	0.00000	0.00000
76	*Culex* sp. 01	0.00021	0.00440	0.00078	0.00045
77	*Culex* sp. 02	0.00000	0.00648	0.00000	0.00000
78	Culicidae 01	0.00000	0.00313	0.00000	0.00000
79	Culicidae 02	0.00000	0.00031	0.00000	0.00000
80	Culicidae 03	0.00000	0.00062	0.00072	0.00030
81	Culicidae 04	0.00000	0.00031	0.00000	0.00000
82	Culicidae 05	0.00015	0.00080	0.00142	0.00187
83	*Cymatia* sp. 01	0.00000	0.00000	0.00072	0.00000
84	*Cyphon* sp. 01	0.00000	0.00000	0.00036	0.00000
85	*Cyphon* sp. 02	0.00000	0.00185	0.00000	0.00077
86	*Cyphon* sp. 03	0.00000	0.00010	0.00000	0.00000
87	*Dilatata dilatata*	0.01483	0.00817	0.00000	0.00000
88	Diptera 01	0.00007	0.00000	0.00000	0.00000
89	Diptera 02	0.00000	0.00036	0.00000	0.00000

90	Diptera 03	0.00007	0.00000	0.00000	0.00000
91	Diptera 04	0.00000	0.00000	0.00000	0.00059
92	*Dugesia* sp.1	0.00255	0.00000	0.00114	0.00662
93	*Dugesia* sp.2	0.02393	0.00000	0.00167	0.00000
94	Dytiscidae 02	0.00000	0.00036	0.00000	0.00000
95	*Dytiscus* sp. 01	0.00007	0.00000	0.00047	0.00000
96	*Enochrus* sp.	0.00000	0.00037	0.00000	0.00000
97	*Erpobdella* sp. 01	0.00066	0.00000	0.00226	0.00000
98	Erpobdellidae 01	0.00063	0.00000	0.00000	0.00000
99	*Erythromma viridulum*	0.00242	0.00000	0.00326	0.01558
100	*Ferrissia californica*	0.00022	0.00024	0.00000	0.00000
101	Gammaridae 01	0.00305	0.00000	0.00207	0.00000
102	Gammaridae 02	0.00097	0.00000	0.00021	0.00099
103	Gastropoda	0.00014	0.00000	0.00000	0.00090
104	*Gerris lacustris*	0.00054	0.00139	0.00046	0.00000
105	*Gerris* sp.1	0.00000	0.00000	0.00000	0.00042
106	*Gerris* sp.2	0.00004	0.00019	0.00014	0.00000
107	*Gerris* sp.3	0.00000	0.00010	0.00087	0.00000
108	*Gerris* sp.4	0.00000	0.00000	0.00043	0.00000
109	*Gerris* sp.5	0.00000	0.00238	0.00375	0.00000
110	*Gerris* sp.6	0.00000	0.00031	0.00000	0.00000
111	*Gerris* sp.7	0.00000	0.00145	0.00000	0.00000
112	Glossiphoniidae 01	0.00000	0.00000	0.00000	0.00147
113	*Gordea* sp. 01	0.00024	0.00000	0.00000	0.00000
114	*Graphoderus cinereus*	0.00000	0.00021	0.00000	0.00000
115	*Graphoderus* sp. 01	0.00006	0.00000	0.00000	0.00000
116	*Gyrinus substriatus*	0.00000	0.00168	0.00050	0.00000
117	*Haliplus flavicollis*	0.00000	0.00000	0.00000	0.00084
118	*Haliplus fulvus*	0.00000	0.00031	0.00000	0.00000
119	*Haliplus heydeni*	0.00028	0.00000	0.00058	0.00699
120	*Haliplus immaculatus*	0.00000	0.00010	0.00036	0.00307
121	*Haliplus lineatocollis*	0.00009	0.00455	0.00386	0.01284
122	*Haliplus obliquus*	0.00000	0.00063	0.00000	0.00506
123	*Haliplus ruficollis*	0.00008	0.00031	0.00425	0.00718
124	*Haliplus* sp. 01	0.00044	0.00000	0.00000	0.00000
125	*Haliplus* sp. 03	0.00147	0.00058	0.00000	0.00090
126	*Haliplus* sp. 04	0.00019	0.00060	0.00000	0.00077
127	*Haliplus* sp. 05	0.00082	0.00036	0.00000	0.00000
128	*Helobdella* sp. 01	0.00000	0.00037	0.00000	0.00000
129	*Helobdella* sp. 02	0.01629	0.00457	0.00985	0.02167
130	*Helochares* sp. 01	0.00000	0.00000	0.00068	0.00000
131	*Helochares* sp. 02	0.00019	0.00000	0.00000	0.00000
132	*Helochares* sp. 03	0.00000	0.00037	0.00000	0.00000
133	*Helophorus* sp. 01	0.00000	0.00000	0.00009	0.00000
134	*Hemiclepsis marginata*	0.00009	0.00000	0.00019	0.00059
135	Hesperocorixa 01	0.00011	0.00083	0.00099	0.00587

136	Hesperocorixa 02	0.00020	0.00256	0.00109	0.00000
137	Hesperocorixa 03	0.00102	0.00368	0.02441	0.01287
138	Hesperocorixa 04	0.00000	0.00047	0.00000	0.00859
139	*Hippeutis complanatus*	0.00700	0.05408	0.00665	0.00927
140	*Hydra* sp. 01	0.00053	0.01718	0.00000	0.00030
141	Hydracari 01	0.00238	0.00000	0.00206	0.00000
142	Hydracari 02	0.00000	0.00000	0.00109	0.00000
143	Hydracari 03	0.00210	0.00016	0.00178	0.00037
144	Hydracari 04	0.00000	0.00208	0.00000	0.00000
145	Hydracari 05	0.00000	0.00055	0.00000	0.00000
146	Hydracari 06	0.00000	0.00000	0.00000	0.00110
147	*Hydraena* sp. 01	0.00000	0.00010	0.00000	0.00000
148	*Hydraena* sp. 02	0.00000	0.00010	0.00000	0.00000
149	*Hydrobius* sp. 01	0.00000	0.00072	0.00000	0.00000
150	*Hydrometra stagnorum*	0.00000	0.00036	0.01510	0.00419
151	Hydrophilidae 01	0.00004	0.00000	0.00000	0.00000
152	Hydroporinae 01	0.00013	0.00000	0.00000	0.00000
153	*Hydroporus angustatus*	0.00041	0.00216	0.00000	0.00000
154	*Hydroporus figuratus*	0.00014	0.00000	0.00000	0.00452
155	*Hydroporus palustris*	0.00025	0.00024	0.00754	0.00725
156	*Hygrobia hermanni*	0.00009	0.00000	0.00000	0.00000
157	*Hygrotus inaequalis*	0.00051	0.00388	0.02378	0.01007
158	*Hyphydrus ovatus*	0.00290	0.00178	0.02369	0.01744
159	*Ischnura* sp.1	0.00284	0.00777	0.00075	0.00591
160	*Ischnura* sp.2	0.00000	0.00163	0.00038	0.00000
161	*Ischnura* sp.3	0.00000	0.00037	0.00056	0.00000
162	*Laccobius* sp. 01	0.00000	0.00000	0.00038	0.00000
163	*Laccophilus minutus*	0.00000	0.00530	0.00000	0.00147
164	*Laccophilus* sp. 01	0.00130	0.00000	0.00053	0.00000
165	*Laccophilus* sp. 02	0.00206	0.00000	0.00000	0.00000
166	*Laccophilus* sp. 03	0.00188	0.00000	0.00000	0.00000
167	*Lestes viridis*	0.00105	0.00050	0.00924	0.00000
168	Libellulidae 01	0.00000	0.00000	0.00000	0.00037
169	Limnephilidae 02	0.00000	0.00000	0.00000	0.00045
170	*Limnoxenus niger*	0.00018	0.00000	0.00000	0.00000
171	*Micronecta* sp. 01	0.00017	0.00000	0.00036	0.00000
172	*Microvelia* sp. 01	0.00009	0.00245	0.00000	0.00042
173	*Microvelia* sp. 02	0.00000	0.00000	0.00046	0.00000
174	*Musculium* sp.2	0.00044	0.00000	0.00000	0.00000
175	*Musculium* sp.3	0.00056	0.00000	0.00920	0.00118
176	*Myxas* sp.	0.00287	0.00000	0.00000	0.00000
177	*Naucoris maculatus*	0.00057	0.00000	0.00449	0.00000
178	*Noterus clavicornis*	0.00000	0.00000	0.00036	0.00042
179	*Notonecta* sp. 01	0.00030	0.00519	0.05028	0.00833
180	*Notonecta* sp. 02	0.00666	0.00000	0.00869	0.00000
181	*Ochthebius minutus*	0.00000	0.00016	0.00000	0.00045

182	*Odontomyia* sp.01	0.00008	0.00000	0.00000	0.00000
183	*Odontomyia* sp.02	0.00004	0.00000	0.00000	0.00000
184	*Oplodontha* sp. 01	0.00000	0.00037	0.00000	0.00000
185	*Oplodontha* sp. 02	0.00057	0.00000	0.00000	0.00000
186	*Orthetrum* sp. 01	0.00000	0.00016	0.00000	0.00000
187	Orthocladiinae 01	0.00575	0.00000	0.00011	0.00000
188	Orthocladiinae 02	0.00632	0.00290	0.00000	0.00000
189	Orthocladiinae 03	0.09402	0.00178	0.00767	0.00000
190	Orthocladiinae 04	0.00209	0.03250	0.00000	0.00000
191	*Peltodytes caesus*	0.00004	0.00000	0.00000	0.00042
192	*Physella acuta*	0.03366	0.00443	0.02411	0.03215
193	*Pisidium* sp.1	0.00068	0.00496	0.01413	0.00000
194	*Pisidium* sp.2	0.00000	0.00000	0.00000	0.00074
195	*Planaria torva*	0.00020	0.04437	0.00000	0.00000
196	*Platycnemis* sp. 01	0.00000	0.00412	0.00000	0.00223
197	*Plea minutissima*	0.00131	0.02655	0.01421	0.01942
198	*Podura aquatica*	0.00000	0.00000	0.00210	0.00202
199	Polycentropodidae 01	0.00007	0.00000	0.00000	0.00000
200	*Potamopyrgus antipodarum*	0.07138	0.00256	0.00589	0.00539
201	*Proasellus* sp.	0.01505	0.04956	0.00906	0.01720
202	*Procambarus clarkii*	0.00000	0.00000	0.00010	0.00000
203	*Pyrrhosoma nymphula*	0.00000	0.00268	0.00046	0.00000
204	*Ranatra linearis*	0.00000	0.00000	0.00038	0.00000
205	*Rhantus suturalis*	0.00000	0.00000	0.00000	0.00090
206	Sciomyzidae 01	0.00006	0.00000	0.00000	0.00000
207	*Segmentina nitida*	0.00199	0.00000	0.00000	0.00000
208	*Sialis* sp. 01	0.00017	0.00013	0.00077	0.00000
209	Sigara 01	0.00027	0.00041	0.00000	0.00119
210	Sigara 02	0.00000	0.00310	0.00000	0.02538
211	Sigara 03	0.00182	0.00219	0.00000	0.01701
212	Sigara 04	0.00028	0.00047	0.00000	0.00000
213	Sigara 06	0.00000	0.00221	0.00318	0.00000
214	*Sympetrum* sp.1	0.00000	0.00000	0.00050	0.00000
215	*Sympetrum* sp.2	0.00000	0.00000	0.00093	0.00000
216	*Sympetrum* sp.3	0.00297	0.00000	0.01068	0.00000
217	Syrphidae 01	0.00000	0.00000	0.00028	0.00000
218	Tabanidae 01	0.00000	0.00010	0.00000	0.00000
219	Tabanidae 02	0.00000	0.00021	0.00000	0.00000
220	Tanypodinae 01	0.00040	0.00000	0.04110	0.00000
221	Tanypodinae 02	0.00383	0.00110	0.04738	0.00000
222	Tanypodinae 03	0.00150	0.00125	0.00000	0.00000
223	Tanypodinae 04	0.00074	0.00978	0.00207	0.00255
224	Tanypodinae 05	0.00000	0.01153	0.00000	0.00000
225	Tanypodinae 06	0.00000	0.00733	0.00000	0.01252
226	Tanypodinae 07	0.00020	0.00072	0.00000	0.00000
227	Tanypodinae 08	0.00000	0.00031	0.00000	0.00000

228	Tanypodinae 09	0.00000	0.00254	0.00452	0.00000
229	Tanypodinae 10	0.00000	0.00691	0.00000	0.00000
230	Tanypodinae 11	0.00000	0.00000	0.00000	0.00845
231	*Tanysphyrus lemnae*	0.00000	0.00108	0.00000	0.00000
232	Tanytarsini 01	0.00320	0.09510	0.00000	0.00000
233	Tanytarsini 02	0.00182	0.00000	0.00000	0.00000
234	*Theremyzon* sp. 01	0.00034	0.00016	0.00029	0.00000
235	Trichoptera 02	0.00000	0.00000	0.00000	0.00089
236	*Valvata macrostoma*	0.05748	0.04464	0.02756	0.07712

Table S7. Results of the redundancy analyses (RDA) parsimonious models based on morphotaxa abundance.

Variables	**Cumulative adjusted R^2**	**F value**	***p* value**
Fungicide concentration in sediment	0.047	3.142	0.001
MTE1	0.079	2.465	0.002
Water conductivity	0.098	1.862	0.014
COD	0.115	1.737	0.024
Pharmaceutical concentration in sediment	0.131	1.748	0.022
Insecticide concentration in water	0.146	1.644	0.034
Organic C concentration in water	0.160	1.601	0.05

Table S8. Morphotaxa scores in the presence-absence-based redundancy analysis (RDA) and the abundance-based RDA.

#	Morphotaxa	Presence-absence-based RDA		Abundance-based RDA	
		RDA1	RDA2	RDA1	RDA2
1	*Acilius* sp.	-0.006	0.002	0.001	0.001
2	*Acilius sulcatus*	-0.007	-0.016	-0.005	-0.013
3	*Acroloxus lacustris*	-0.061	-0.055	-0.112	0.024
4	*Aedes* sp.01	-0.009	-0.011	-0.003	-0.012
5	*Aeshna cyanea*	0.043	0.077	0.029	-0.026
6	*Aeshna grandis*	-0.002	-0.003	-0.002	-0.001
7	*Aeshna* sp. 01	-0.004	-0.003	0.012	-0.056
8	*Aeshna* sp. 02	0.019	0.027	0.002	0.012
9	*Aeshna* sp. 03	0.023	-0.009	0.009	0.019
10	*Aeshna* sp. 04	-0.009	0.033	-0.029	0.022
11	*Aeshna* sp. 05	-0.005	0.003	0.006	-0.015
12	*Aeshna* sp. 06	0.032	0.026	0.023	0.005
13	*Aeshna* sp. 07	-0.010	0.021	0.022	-0.052
14	*Alboglossiphonia hyalina*	-0.016	-0.015	-0.028	0.009
15	*Anacaena* sp. 01	0.000	0.008	0.005	-0.008
16	*Anacaena* sp. 02	-0.004	-0.003	0.003	-0.003
17	*Anophelinae* 01	0.095	-0.152	0.117	0.008
18	*Armiger crista*	-0.063	0.013	-0.088	-0.009
19	*Asellus* sp.	-0.416	-0.198	-0.648	0.124
20	*Baetidae* 02	-0.066	0.194	-0.193	0.313
21	*Batracobdella* sp. 01	0.070	-0.016	0.020	0.069
22	*Berosus affinis*	0.009	-0.004	0.006	0.010
23	*Berosus signaticollis*	-0.005	-0.014	-0.004	-0.006
24	*Berosus* sp. 01	-0.001	0.008	0.003	-0.001
25	*Boreobdella* sp. 01	-0.002	-0.003	0.001	0.004
26	*Bythinia* sp. 01	-0.009	-0.005	-0.017	0.010
27	*Bythinia* sp. 02	-0.004	-0.004	-0.008	0.003
28	*Caenis* sp. 01	-0.002	0.002	0.003	-0.002
29	*Caenis* sp. 02	0.002	-0.024	0.002	-0.004
30	Ceratopogoninae 01	-0.044	0.000	0.032	-0.102
31	Ceratopogoninae 02	-0.087	-0.257	-0.001	-0.150
32	Ceratopogoninae 03	-0.002	-0.005	0.000	-0.004
33	*Ceriagrion* sp 01	-0.023	-0.070	-0.019	-0.030
34	Chaoboridae 01	-0.014	-0.035	-0.014	-0.023
35	*Chaoborus* sp. 01	-0.168	0.063	-0.177	-0.319
36	*Chaoborus* sp. 02	-0.220	0.014	-0.259	-0.287
37	*Chaoborus* sp. 03	-0.036	-0.056	-0.009	-0.061
38	*Chaoborus* sp. 04	-0.006	0.020	-0.010	0.001
39	*Chaoborus* sp. 06	-0.003	-0.010	0.000	-0.008
40	Chironomidae 01	0.004	0.001	0.000	0.004

41	Chironomidae 02	0.003	0.007	0.001	0.010
42	Chironomidae 03	0.007	-0.009	0.005	0.003
43	Chironomidae 04	-0.006	-0.008	-0.013	0.001
44	Chironomidae 05	-0.010	0.054	0.030	-0.006
45	Chironomidae 06	0.000	0.009	0.001	-0.004
46	Chironomidae 07	0.000	0.008	0.005	-0.008
47	Chironomidae 08	0.000	0.012	0.008	-0.011
48	Chironomidae 11	0.019	-0.036	0.011	0.002
49	Chironomini 01	-0.009	-0.026	-0.005	0.005
50	Chironomini 02	-0.001	0.010	0.004	-0.002
51	Chironomini 03	-0.001	-0.001	-0.002	0.000
52	Chironomini 04	-0.011	0.199	0.112	-0.070
53	*Chironomus* sp. 01	-0.076	0.161	0.162	-0.236
54	*Chironomus* sp. 02	-0.069	-0.156	0.059	-0.234
55	*Chironomus* sp. 03	-0.003	-0.002	0.004	-0.001
56	*Chironomus* sp. 04	-0.013	0.033	0.000	0.002
57	*Chironomus* sp. 05	-0.014	0.009	-0.002	0.010
58	*Chironomus* sp. 06	-0.004	-0.001	0.000	0.001
59	Clitellata 01	0.147	0.089	0.055	0.204
60	*Cloeon dipterum*	0.453	-0.401	0.365	0.078
61	*Coenagrion scitulum*	0.040	0.028	-0.002	0.039
62	*Coenagrion* sp.01	0.122	-0.080	0.045	0.078
63	*Coenagrion* sp.02	0.030	0.018	-0.001	0.029
64	Coenagrionidae 01	0.007	-0.011	0.011	0.002
65	Coenagrionidae 02	-0.008	-0.019	0.013	-0.024
66	Coleoptera 02	-0.001	0.021	0.002	-0.010
67	*Colymbetes fuscus*	0.009	-0.015	0.009	-0.002
68	*Colymbetes* sp. 01	-0.002	0.014	-0.006	0.004
69	*Corixa panzeri*	0.084	0.043	0.022	0.106
70	*Corixa punctata*	0.015	0.080	0.010	0.079
71	*Corixa* sp.01	0.026	0.021	0.001	0.022
72	Corixinae 01	-0.015	0.042	-0.041	0.023
73	Corixinae 02	-0.004	0.019	0.050	0.013
74	Corixinae 03	-0.001	-0.004	0.000	-0.006
75	Crambidae	-0.001	0.035	0.023	-0.032
76	*Culex* sp. 01	0.012	-0.087	0.007	-0.012
77	*Culex* sp. 02	-0.021	-0.069	0.004	-0.047
78	Culicidae 01	-0.014	-0.046	0.003	-0.030
79	Culicidae 02	-0.004	-0.015	0.001	-0.009
80	Culicidae 03	0.031	-0.020	0.013	0.017
81	Culicidae 04	-0.001	-0.005	0.000	-0.007
82	Culicidae 05	0.029	-0.060	0.021	-0.001
83	*Cymatia* sp. 01	0.034	-0.012	0.016	0.027
84	*Cyphon* sp. 01	0.024	-0.008	0.012	0.019
85	*Cyphon* sp. 02	0.013	-0.009	0.016	0.005
86	*Cyphon* sp. 03	0.000	-0.003	0.000	-0.004

87	*Dilatata dilatata*	-0.031	-0.040	-0.059	-0.003
88	Diptera 01	-0.001	-0.001	-0.002	0.000
89	Diptera 02	0.000	0.008	0.005	-0.008
90	Diptera 03	-0.001	-0.001	-0.002	0.000
91	Diptera 04	-0.003	-0.002	-0.005	0.001
92	*Dugesia* sp.1	0.067	0.017	-0.010	0.091
93	*Dugesia* sp.2	0.145	-0.005	0.029	0.105
94	Dytiscidae 02	0.000	0.008	0.005	-0.008
95	*Dytiscus* sp. 01	-0.002	0.009	-0.007	0.005
96	*Enochrus* sp.	0.007	-0.009	0.005	0.003
97	*Erpobdella* sp. 01	-0.011	0.007	0.015	-0.001
98	Erpobdellidae 01	-0.005	-0.006	0.004	0.001
99	*Erythromma viridulum*	0.094	-0.144	0.031	0.041
100	*Ferrissia californica*	-0.006	-0.011	-0.008	-0.004
101	Gammaridae 01	-0.013	-0.002	-0.030	0.015
102	Gammaridae 02	-0.014	-0.010	-0.023	0.003
103	Gastropoda	-0.003	0.010	-0.007	0.003
104	*Gerris lacustris*	-0.016	-0.027	-0.004	-0.025
105	*Gerris* sp.1	-0.005	-0.014	-0.004	-0.006
106	*Gerris* sp.2	-0.005	-0.008	-0.006	-0.002
107	*Gerris* sp.3	-0.008	-0.007	0.000	-0.014
108	*Gerris* sp.4	0.022	-0.011	0.010	0.018
109	*Gerris* sp.5	-0.009	-0.008	0.008	-0.027
110	*Gerris* sp.6	-0.001	-0.005	0.000	-0.007
111	*Gerris* sp.7	-0.001	-0.011	-0.001	-0.016
112	Glossiphoniidae 01	0.017	0.006	0.001	0.012
113	*Gordea* sp. 01	-0.002	0.005	0.001	-0.001
114	*Graphoderus cinereus*	-0.001	-0.004	0.000	-0.006
115	*Graphoderus* sp. 01	0.000	0.003	0.000	-0.001
116	*Gyrinus substriatus*	-0.008	-0.004	-0.011	-0.002
117	*Haliplus flavicollis*	-0.007	-0.020	-0.005	-0.009
118	*Haliplus fulvus*	-0.004	-0.015	0.001	-0.009
119	*Haliplus heydeni*	-0.005	0.031	0.004	-0.006
120	*Haliplus immaculatus*	0.021	0.012	0.020	0.014
121	*Haliplus lineatocollis*	0.059	-0.050	0.021	0.036
122	*Haliplus obliquus*	-0.023	-0.070	-0.012	-0.035
123	*Haliplus ruficollis*	-0.039	-0.084	-0.009	-0.059
124	*Haliplus* sp. 01	0.008	0.010	0.000	0.005
125	*Haliplus* sp. 03	-0.016	-0.010	0.002	0.002
126	*Haliplus* sp. 04	-0.006	-0.008	0.005	-0.012
127	*Haliplus* sp. 05	0.021	-0.015	0.015	0.001
128	*Helobdella* sp. 01	-0.003	-0.003	-0.005	0.001
129	*Helobdella* sp. 02	0.007	0.097	-0.109	0.160
130	*Helochares* sp. 01	-0.001	0.012	0.005	0.003
131	*Helochares* sp. 02	0.007	-0.012	0.005	0.001
132	*Helochares* sp. 03	0.007	-0.009	0.005	0.003

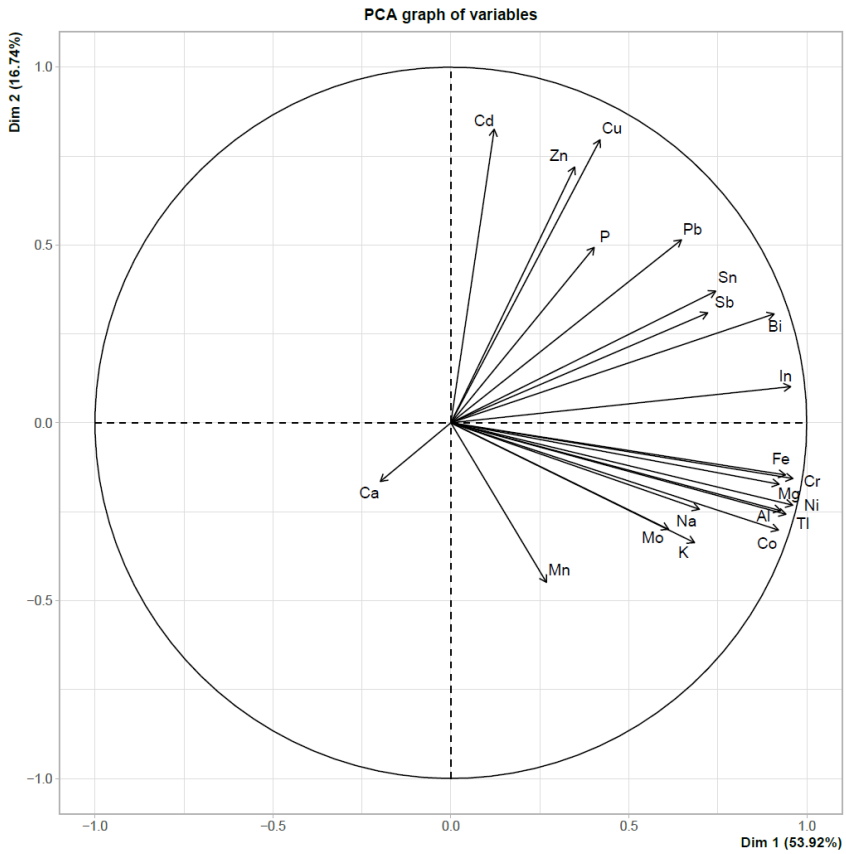
133	*Helophorus* sp. 01	-0.001	-0.001	-0.003	0.001
134	*Hemiclepsis marginata*	0.000	-0.012	-0.005	0.003
135	Hesperocorixa 01	0.007	-0.003	-0.008	0.007
136	Hesperocorixa 02	0.057	-0.043	0.031	0.036
137	Hesperocorixa 03	-0.118	-0.097	-0.091	-0.068
138	Hesperocorixa 04	-0.019	-0.034	-0.012	-0.022
139	*Hippeutis complanatus*	-0.088	-0.064	-0.177	0.063
140	*Hydra* sp. 01	-0.017	-0.006	-0.042	0.033
141	Hydracari 01	0.002	0.040	-0.013	0.025
142	Hydracari 02	-0.009	-0.014	0.010	-0.023
143	Hydracari 03	0.084	0.000	0.023	0.058
144	Hydracari 04	0.002	0.007	-0.002	0.014
145	Hydracari 05	0.002	0.002	0.000	0.003
146	Hydracari 06	-0.002	0.020	0.001	0.002
147	*Hydraena* sp. 01	0.000	-0.003	0.000	-0.004
148	*Hydraena* sp. 02	0.000	-0.003	0.000	-0.004
149	*Hydrobius* sp. 01	0.000	0.012	0.008	-0.011
150	*Hydrometra stagnorum*	-0.014	0.102	0.027	-0.096
151	Hydrophilidae 01	-0.002	-0.005	0.000	-0.004
152	Hydroporinae 01	-0.003	-0.004	-0.001	0.004
153	*Hydroporus angustatus*	-0.001	0.034	0.014	-0.025
154	*Hydroporus figuratus*	-0.005	0.025	-0.012	0.006
155	*Hydroporus palustris*	-0.035	0.048	-0.020	-0.036
156	*Hygrobia hermanni*	0.005	-0.009	0.003	0.000
157	*Hygrotus inaequalis*	-0.018	0.158	0.024	-0.140
158	*Hyphydrus ovatus*	-0.063	0.098	0.006	-0.125
159	*Ischnura* sp.1	0.041	-0.045	0.032	0.025
160	*Ischnura* sp.2	-0.010	0.000	0.010	0.005
161	*Ischnura* sp.3	0.017	0.001	0.003	0.004
162	*Laccobius* sp. 01	-0.002	0.002	0.003	-0.002
163	*Laccophilus minutus*	0.043	-0.017	0.021	0.033
164	*Laccophilus* sp. 01	0.027	-0.041	0.023	-0.006
165	*Laccophilus* sp. 02	-0.004	-0.012	0.005	0.022
166	*Laccophilus* sp. 03	-0.008	-0.012	0.005	0.017
167	*Lestes viridis*	0.053	-0.089	0.053	0.007
168	Libellulidae 01	0.001	0.008	-0.003	0.009
169	Limnephilidae 02	-0.001	0.008	-0.003	0.002
170	*Limnoxenus niger*	-0.001	0.006	0.002	-0.001
171	*Micronecta* sp. 01	0.023	-0.008	0.008	0.021
172	*Microvelia* sp. 01	0.002	-0.056	0.004	-0.030
173	*Microvelia* sp. 02	-0.005	-0.011	0.008	-0.014
174	*Musculium* sp.2	-0.004	-0.003	-0.007	0.000
175	*Musculium* sp.3	0.008	0.055	0.029	0.009
176	*Myxas* sp.	-0.008	-0.007	0.006	-0.007
177	*Naucoris maculatus*	-0.003	0.005	0.016	-0.008
178	*Noterus clavicornis*	0.019	-0.023	0.008	0.013

179	*Notonecta* sp. 01	-0.027	0.101	0.050	0.035
180	*Notonecta* sp. 02	0.053	-0.008	0.001	0.071
181	*Ochthebius minutus*	-0.004	-0.002	-0.003	-0.005
182	*Odontomyia* sp.01	-0.002	-0.007	0.000	-0.005
183	*Odontomyia* sp.02	-0.002	-0.005	0.000	-0.004
184	*Oplodontha* sp. 01	-0.003	-0.003	-0.004	-0.001
185	*Oplodontha* sp. 02	-0.004	-0.003	0.003	-0.003
186	*Orthetrum* sp. 01	-0.003	-0.010	0.001	-0.007
187	Orthocladiinae 01	0.055	-0.008	0.013	0.059
188	Orthocladiinae 02	0.006	-0.020	0.043	-0.002
189	Orthocladiinae 03	0.056	-0.094	0.127	0.091
190	Orthocladiinae 04	-0.052	-0.076	0.046	-0.015
191	*Peltodytes caesus*	-0.006	-0.020	-0.004	-0.010
192	*Physella acuta*	-0.182	-0.115	-0.118	-0.089
193	*Pisidium* sp.1	0.040	0.042	0.060	-0.020
194	*Pisidium* sp.2	0.002	0.012	-0.004	0.013
195	*Planaria torva*	0.004	0.030	-0.024	0.070
196	*Platycnemis* sp. 01	-0.004	0.031	0.020	0.024
197	*Plea minutissima*	0.086	-0.357	0.112	-0.082
198	*Podura aquatica*	-0.002	0.049	0.009	-0.039
199	Polycentropodidae 01	-0.001	-0.001	-0.001	0.000
200	*Potamopyrgus antipodarum*	-0.143	-0.343	-0.054	-0.223
201	*Proasellus* sp.	-0.128	-0.114	-0.227	0.052
202	*Procambarus clarkii*	-0.002	-0.003	-0.002	-0.001
203	*Pyrrhosoma nymphula*	-0.015	-0.036	0.012	-0.057
204	*Ranatra linearis*	-0.002	0.002	0.003	-0.002
205	*Rhantus suturalis*	-0.001	0.012	-0.005	0.003
206	Sciomyzidae 01	0.000	0.003	0.000	-0.001
207	*Segmentina nitida*	-0.002	0.020	0.000	-0.006
208	*Sialis* sp. 01	-0.006	0.011	-0.009	0.007
209	Sigara 01	-0.002	-0.019	-0.007	-0.006
210	Sigara 02	-0.020	0.037	-0.032	0.075
211	Sigara 03	-0.034	-0.013	-0.006	0.039
212	Sigara 04	-0.008	-0.023	0.003	-0.005
213	Sigara 06	-0.031	-0.047	-0.017	-0.027
214	*Sympetrum* sp.1	-0.003	0.002	-0.001	-0.004
215	*Sympetrum* sp.2	-0.007	-0.016	0.011	-0.020
216	*Sympetrum* sp.3	0.058	-0.069	0.070	-0.007
217	Syrphidae 01	-0.002	-0.001	-0.003	0.001
218	Tabanidae 01	0.000	-0.003	0.000	-0.004
219	Tabanidae 02	-0.001	-0.004	0.000	-0.006
220	Tanypodinae 01	-0.029	0.120	0.031	-0.039
221	Tanypodinae 02	-0.025	0.009	0.056	-0.005
222	Tanypodinae 03	0.000	-0.037	0.013	-0.020
223	Tanypodinae 04	-0.002	-0.038	0.064	-0.079
224	Tanypodinae 05	-0.002	0.047	0.031	-0.042

225	Tanypodinae 06	-0.017	0.028	0.000	-0.019
226	Tanypodinae 07	-0.001	0.021	0.008	-0.015
227	Tanypodinae 08	-0.004	-0.015	0.001	-0.009
228	Tanypodinae 09	-0.018	0.022	0.000	0.000
229	Tanypodinae 10	0.009	-0.001	0.036	-0.035
230	Tanypodinae 11	-0.004	0.039	0.014	-0.001
231	*Tanysphyrus lemnae*	-0.001	0.014	0.009	-0.013
232	Tanytarsini 01	-0.053	0.014	0.078	0.049
233	Tanytarsini 02	-0.007	0.004	0.006	-0.010
234	*Theremyzon* sp. 01	-0.006	-0.002	0.000	-0.008
235	Trichoptera 02	-0.004	-0.002	-0.006	0.001
236	*Valvata macrostoma*	0.495	0.153	0.105	0.459

Figure S1. Principal component analysis (PCA) in all ponds and in the four field campaigns with trace elements (TE). A. Correlation circle; B. Graph of the ponds.

A.



B.

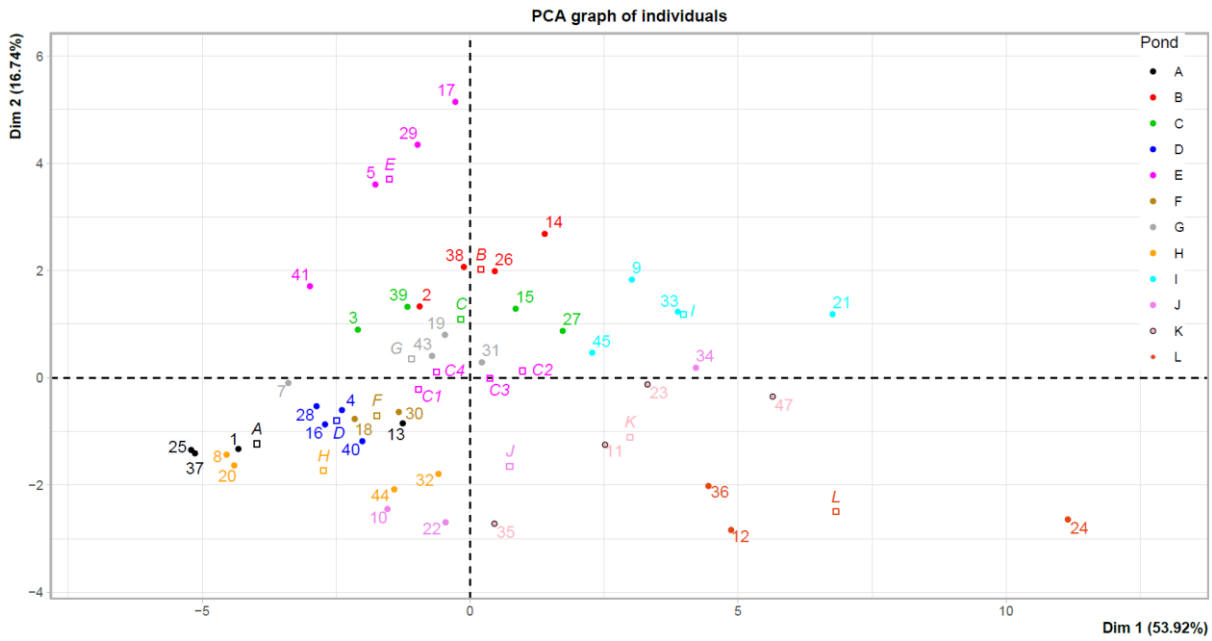
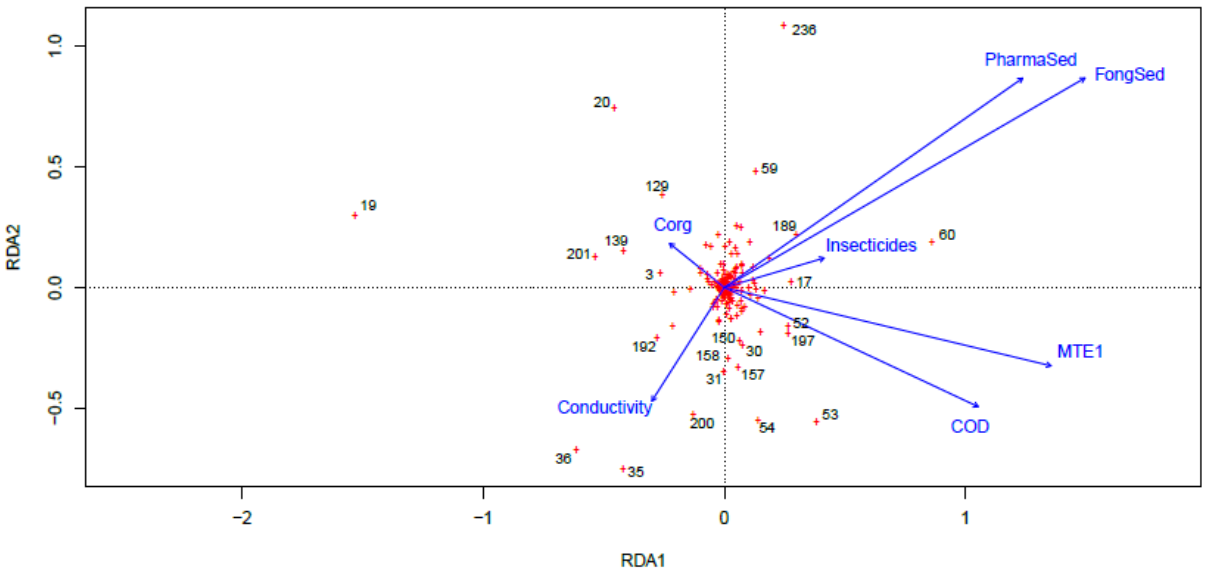
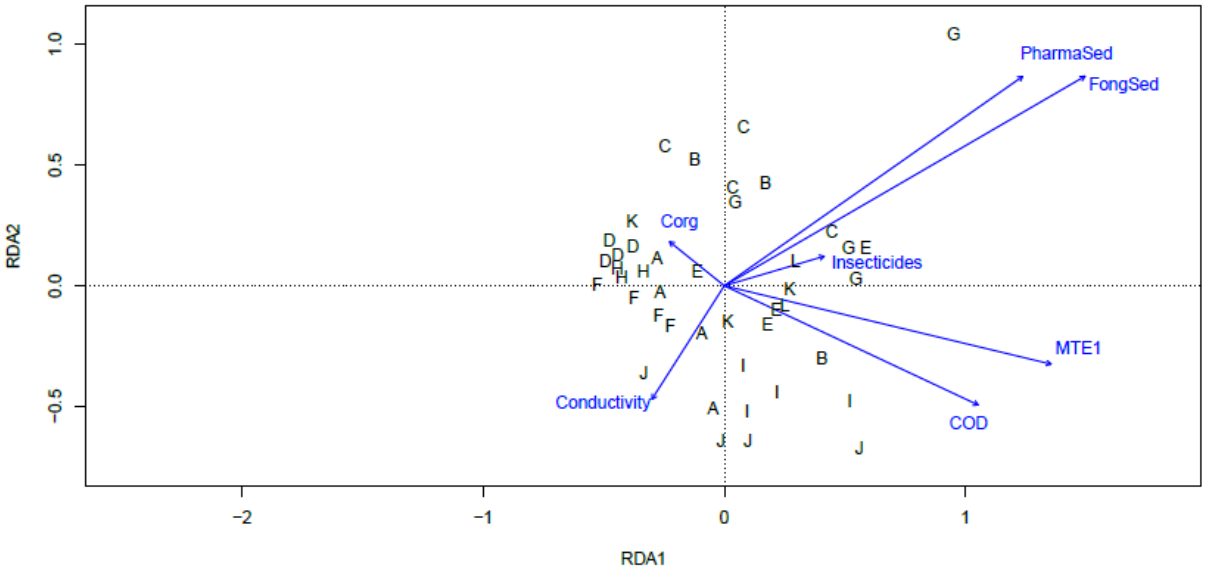


Figure S2. Parsimonious redundancy analysis (RDA) based on morphotaxa abundances. The biplots show the variables (blue arrows) with either the morphotaxa (numbers, panel a) or the ponds (letters, panel b). The number of morphotaxa is shown in brackets: *Acroloxus lacustris* (3); *Asellus* sp. (19); Baetidae 02 (20); Ceratopogoninae 01 (30); Ceratopogoninae 02 (31); *Chaoborus* sp. 01 (35); *Chaoborus* sp. 02 (36); *Chironomus* sp. 01 (53); *Chironomus* sp. 02 (54); Clitellata 01 (59); *Cloeon dipterum* (60); *Helobdella* sp. 02 (129); *Hippeutis complanatus* (139); *Hydrometra stagnorum* (150); *Hygrotus inaequalis* (157); *Hyphydrus ovatus* (158); Orthocladiinae 03 (189), *Physella acuta* (192); *Potamopyrgus antipodarum* (200); *Proasellus* sp. (201); and *Valvata macrostoma* (236). COD: chemical oxygen demand; Conductivity: water conductivity; Corg: organic carbon concentration in water; FongSed: Fungicide concentration in sediment; Insecticides: Insecticide concentration in water; MTE1: Trace element in water – PCA axis 1; PharmaSed: concentration of pharmaceuticals in sediment.


PharmaSed
FongSed
Corg
Insecticides
MTE1
COD
Conductivity
RDA1
RDA2


PharmaSed
FongSed
Corg
Insecticides
MTE1
COD
Conductivity
RDA1
RDA2