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Metal bioavailable contamination engages richness decline, species turnover but unchanged functional diversity of stream macroinvertebrates at the scale of a French region

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Abstract

Freshwater ecosystems are the main source of water for sustaining life on earth, and the biodiversity they support is the main source of valuable goods and services for human populations. Despite growing recognition of the impairment of freshwater ecosystems by micropollutant contamination, different conceptual and methodological considerations can newly be addressed to improve our understanding of the ecological impact into these ecosystems. Here, we originally combined in situ ecotoxicology and community ecology concepts to unveil the mechanisms structuring macroinvertebrate communities along a regional contamination gradient. For this, our study benefited from the recent deployment of an innovative biomonitoring approach relying on the measurement of metal bioaccumulation in caged crustaceans throughout the French river monitoring network. We were hence able to identify 23 streams presenting a significant gradient of bioavailable metal contamination within the same catchment area in the South West of France, from which we also obtained data on the composition of resident macroinvertebrate communities. Analyses of structural and functional integrity of communities revealed an unexpected decoupling between taxonomic and functional diversity of communities in response to bioavailable metal contamination. We show that despite the negative impact of bioavailable metal contamination exposure on taxonomic diversity (with an average species loss of 17% in contaminated streams), functional diversity is maintained through a process of non-random species replacement by functional redundant species at the regional scale. Such unanticipated findings call for a deeper characterization of metal-tolerant communities' ability to cope with environmental variability in multi-stressed ecosystems.

Keywords

freshwater macroinvertebrate, taxonomic diversity, functional diversity, functional redundancy, micropollutant contamination, biomonitoring.

1. Introduction

With growing human population comes an increase in the diversity, production (e.g., industrial activities, mining) and release (e.g., urban or agricultural runoff) of chemicals into freshwater ecosystems (Paul and Meyer, 2001; Carpenter et al., 2011). Hence chemicals have become a major component of global environmental change, in the same level of concern as other drivers such as atmospheric CO2 concentration (Bernhardt et al., 2017). Until recently, this global dimension of chemical contamination was not well appreciated (e.g., Steffen et al., 2015; Persson et al., 2022), which may partly explain why micropollutant pollution is one of the least-studied stressors in freshwater ecology (Hevia et al., 2016). Among all micropollutants, trace metals are harmful environment pollutants owing to their toxicity, non-biodegradability, and bioaccumulation (Hare, 1992; Stankovic et al., 2014).

Metal contamination can affect the health of ecosystems throughout adverse effects on population dynamics and long-term adaptability of biological communities (Ricciardi et al., 2009; Pereira et al., 2012). Field observations indicate that stream macroinvertebrate assemblages are sensitive to metal contamination (e.g., Clements et al., 2000), while they are a critical component for regulating the functioning of these ecosystems (Vannote et al., 1980; Wallace and Webster, 1996). Typical documented modifications include reduced macroinvertebrate abundance, reduced species richness, and shifts in community composition from sensitive to tolerant taxa (e.g., Gower et al., 1994; Clements and Kiffney, 1995; Maret et al., 2003; Cain et al., 2004; Liess et al., 2017; Costas et al., 2018). Relationships have also been shown between metal contamination levels in biota and ecological impacts (i.e., abundance) on freshwater communities (e.g., Rainbow et al., 2012).

Beyond these shifts in taxonomic composition (structural component), community integrity also has a functional component, and both must be considered simultaneously when assessing the impairment of macroinvertebrate communities (Minshall 1996). Functional integrity can be assessed via the measure of functional diversity from a multiple-trait-based approach (Statzner at al., 2001). The rationale for the complementary measure of functional diversity is that it provides clearer mechanistic links to ecosystem services, since species traits are the main properties by which organisms influence ecosystem processes (Petchey and Gaston, 2006; Gagic et al., 2015). In the simplest scenario, changes in both taxonomic and functional diversity are congruent (Brice et al., 2017; Jarzyna and Jetz, 2017). But they can also diverge from this expectation with a decoupling between both (Purschke et al., 2013; Robroek et al., 2017) as a result of high functional distinctness between species (Sonnier et al., 2014; White et al., 2018) or high functional similarity (i.e., functional redundancy; Villéger et al., 2014; Mori et al., 2015). The evaluation of these alternative co-variation patterns between functional and taxonomic diversity is essential to predict the future ecological trajectories of ecosystems in response to anthropogenic pressures including micropollutant contamination.

On a more methodological point of view, the level of metal exposure of aquatic communities is usually estimated from chemical measurements in the water column and/or sediments in the majority of field studies. Detecting chemicals in these environmental compartments does not integrate the bioavailability of contaminants, which conditions harmful effects on organisms. That is why the European Water Framework Directive (WFD) increasingly integrates contaminant measurements in biota (Wernersson et al., 2015). This approach displays several advantages: (i) the bioconcentration of contaminants by organisms leads to easier and accurate quantifications of micropollutants; (ii) these measurements reflect the bioavailable fraction (potentially toxic); (iii) they constitute time-integrated measurements (Besse et al., 2012; Inostroza et al., 2016). Measurements in biota can be applied in passive biomonitoring approach, using indigenous organisms (e.g., Kraak et al., 1991; Caçador et al., 2012; Bertrand et al., 2018), or in active biomonitoring approach, using field-caged organisms originated from natural or farmed reference populations (e.g., Mersch et al., 1996; Oikari, 2006; Prygiel et al., 2016). Active biomonitoring approach is based on "standardized" organisms (gender, size, physiological status) from a reference population, allowing like this to limit the impact of biological confounding factors known to modulate accumulation (Besse et al., 2012). Moreover, this approach allows to control exposure time (history), providing a reliable comparison of measured bioavailable contamination levels in space and time (between sampling events; Burton et al., 2005; Shahid et al., 2018). The caging methodology proposed by Besse et al. (2013) with the sentinel species Gammarus fossarum has been recently adapted and standardized by the French agency AFNOR (AFNOR, 2019) and then was used by French water agencies for the national chemical survey of waterbodies. Over the period from 2009 to 2016, this biomonitoring tool has been deployed at more than 200 stations (Alric et al., 2019) belonging to two major monitoring networks established by French water agencies implementing the WFD requirements, the surveillance monitoring network and the operational monitoring network, on which 18 metals and 43 persistent organics pollutants (POPs) have been researched, including priority substances of the WFD (European Commission, 2008). From this database of French water agencies, Alric et al. (2019) developed multi-substance indicators (IBC) to estimate the overall contamination level in aquatic ecosystems. One indicator was developed for metals (IBC_{metals}) and another for POPs (IBC_{POPs}). The strength of such an indicator lies in the fact that it represents the bioavailable contamination fraction, is integrative and allows a reliable comparison of contamination levels in space and time, and thus offers the opportunity to question/study the community response to environmental contamination. In addition, Alric et al. (2019) observed a relationship between bioavailable metal contamination and reduction of abundance, at the national scale, of a few sensitive macroinvertebrate families.

The aim of this study is to investigate alterations in the integrity (i.e., structural and functional) of macroinvertebrate communities in response to bioavailable metal contamination at a regional scale. This purpose was possible by using the database built by the French water agencies through the national chemical biomonitoring survey of waterbodies with caged G. fossarum and previously exploited and presented by Alric et al. (2019). Our goal was (i) to evaluate detailed changes in both taxonomic and functional diversity in response to a metal contamination gradient, (ii) to examine the congruence or divergence of these changes and (iii) to identify underlying ecological processes. Hence, this regional study made it possible to understand changes in the composition of communities by testing the hypotheses of erosion (i.e., simple loss with selection of resistant) vs species replacement and to interpret the modifications of local species assemblages in terms of functions potentially realized in stream ecosystems.

2. Materials and Methods

2.1. Datasets

To investigate the impact of metal chemical pressure on the integrity (structural and functional) of stream macroinvertebrate we used data extracted from the database of the national chemical survey conducted by the French water agencies and presented by Alric et al. (2019). The objective here was to have sites exhibiting similar contextual characteristics (watershed, size range, elevation range) and showing a gradient of bioavailable metal contamination. Twenty-three sites meeting these requirements were selected, located in the Adour-Garonne catchment (the South West of France) which drains an area of 117,650 km2. These sites have comparable environmental settings with stream elevations ranging from 9 to 493 m, stream sizes (stream order) according to the Strahler number ranging from 1 to 5 and streams locations (identity of the hydroecoregion according to Wasson et al., 2002) mainly in lowland and small relief (Table 1). For each sampling site, 20 physico-chemical parameters (Table 1; Table S1) were available and will be used in order to subsequently test for potential confounding effects on the structural integrity of macroinvertebrate communities. These sites also exhibited a gradient of metal contamination (Table 1) and were free for POPs followed by the French water agencies in the biota support, namely PAHs, PCBs, and organochlorine pesticides. Given that study sites are subject to temporal survey of macroinvertebrates as part of the monitoring programs implemented in France by the WFD, the information on macroinvertebrates communities (abundance of taxa) was extracted from the national database “naiades” (http://www.naiades.eaufrance.fr) according to a temporal matching with dates of caging. Standardized protocols, following the recommendations of national and international standards, are used to sample macroinvertebrates annually between May and October (multi-habitat sampling protocol; AFNOR, 2009) and to characterize benthic macroinvertebrate assemblages (AFNOR, 2010; Table S2).

2.2. Metrics of taxonomic diversity

A species accumulation curve was calculated to estimate whether the sampling effort realized in the considered regional area (i.e., the spatial extent covered by the 23 sites) was sufficient to collect most of the species occurring. The accumulation curve was calculated from 9,999 random permutations of all the sampling sites constituting our dataset without replacement (Gotelli and Colwell, 2001) and by using the jackknife estimator (here of the order 1) to estimate the species richness. The jackknife estimator presents the advantage to provide reliable estimation of richness even though the sampling effort is low (Brose et al., 2006). The accumulation of local diversity, described by the species accumulation curve, allows to infer the sample completeness for a given number of investigated sites. The structural diversity within each sampling site (i.e., the alpha diversity) was calculated using the Shannon diversity index based on the Shannon entropy with a logarithm to the base 2 (Material S1). The Hill number of order 1, corresponding to the exponential of the Shannon entropy with a natural logarithm (Jost, 2006), was also calculated (Material S1) as well as the species richness.

The overall beta diversity, which refers to site-to-site composition dissimilarity, was estimated with the Sørensen dissimilarity index based on incidence data (β_{sor}) and partitioned into two components following the method proposed by Baselga (2010) to assess if beta diversity patterns were structured more through spatial turnover (β_{sim}) or nestedness of species assemblages (β_{nes}) (two patterns relying on deterministic processes). A typical spatial species turnover pattern occurs when species at one site are replaced by different species at another site. Nestedness occurs when the species pool present in one site is a subset of that of another more species-rich site. The characterization of the overall beta diversity was also realized by computing the Bray-Curtis index of abundance-based dissimilarity (β_{BC}) and partitioning it into two components, either balanced variation in species abundances (β_{BC-bal}) or abundance gradients (β_{BC-gra}) (Baselga, 2013). Balanced variation in abundances occurs when the individuals of some species in one site are substituted by the same number of individuals of different species in another site, whereas abundance gradients occur when the number of individuals of all species change from one site to the other with the same sign. In the same way that β_{BC} reduces to β_{sor} when incidence data are used, β_{BC-bal} and β_{BC-gra} are analogous to β_{sim} and β_{nes} respectively (Baselga, 2013).

Metrics were computed in the R software (R Core Team, 2020) using the R-package vegan (Oksanen et al., 2019) whom the specaccum() function to compute the species accumulation curve, the prestonfit() and veiledspec() functions to infer the species richness, and the diversity() function to estimate the Shannon entropy. The beta.pair() and beta.pair.abund() functions in the R-package betapart (Baselga et al., 2018) were used to compute the overall beta diversity and partition it from the incidence and abundance data respectively.

2.3. Metrics of functional structure

Functional diversity of each macroinvertebrate assemblage was based on a species × traits matrix compiled from Usseglio-Polatera et al. (2000) and Tachet et al. (2010). Taxa were characterized by 60 categories of eleven biological traits (Table S3), which were found to be sensitive to micropollutant contamination (Archaimbault et al., 2010). To translate available autoecological information about selected traits into numerical values, a score was assigned to each taxon describing its affinity for that category using a fuzzy-coding technique (Chevenet et al., 1994). Scores range from 0 to 5 indicating no affinity and high affinity of the taxon for a given trait modality, respectively.

Based on the species × traits matrix and the relative abundance of each species, the Rao's quadratic entropy (FD_Q) was computed to estimate the functional diversity of macroinvertebrates assemblages using the modified formula proposed by Champely and Chessel (2002) (Material S2). Given that each trait modality exhibited continuous values, the functional distances matrix between each pair of species used in FD_Q was computed using Euclidean distance. The rational choice to use FD_Q is provided in Material S2. To visualize the relative position of macroinvertebrate species in the functional space, a Principal Coordinates Analysis (PCoA) was then carried out on the functional distance matrix (Euclidean distance) using the pcoa() function of the R-package ape (Paradis and Schielp, 2018). The first two axes were selected as their explained variance (PC1: 12% and PC2: 9%) was greater than the null expectation of a broken stick model (Legendre and Legendre, 2012).

To propose a comprehensive analysis of the response of macroinvertebrate assemblages to metal contamination, we focused on dominant species (in term of abundance) because they are typically drivers of ecosystem processes (Moore, 2006). Dominant species are defined as species having values of $1 - scarcity$ (Material S3) higher than the 40%-quantile of the entire species pool of interest using the scarcity() function of the R-package funrar (Grenié et al., 2017). Each species contributes differently to the trait space occupied by their community. Different metrics, including contribution and originality (Material S3), have been developed to quantify how dissimilar is a species from all others within a community and hence how much it adds to the trait space occupied by the community. We therefore estimated the contribution and originality of dominant species using the eponymous functions in the R-package BAT (Cardoso et al., 2021). We also estimated beta functional diversity among dominant species by decomposing diversity according to the Rao's apportionment quadratic entropy framework (Pavoine et al., 2016) using the EqRS() function of the R-package adiv (Pavoine, 2020), with eq, normed1 or normed2 as consecutive normalizations.

2.4. Data analysis

To test whether alpha diversity and metal contamination were associated, the Shannon diversity values (Hill number of order 1 and species richness) were modelled as a function of the contamination levels (expressed as the values of IBC_{metals}) using a quantile regression analyses and the R-package quantreg (Koenker, 2018). Quantile regression coefficients were estimated for percentile (τ) equal to 90%. Linear and curvilinear (exponential) models were built and selection procedure based on Akaike Information Criteria (AIC) was used to determine which model (linear or curvilinear) best fits the data.

Following a null model approach, we tested whether the observed value of overall beta diversity (i.e., β_{BC}, β_{sor}) differed from the value resulting to random species assembly. The null distribution of beta diversity was estimated by generating 9,999 random assemblage matrices according to the fixed-fixed (FF) null model (Material S4). Depending on the trade-off of not being too prone to both Type I (rejecting a true null hypothesis) and Type II (failing to reject a false null hypothesis) statistical errors, this null model is fairly robust compared to other null models (Gotelli and Ulrich, 2012; Ulrich and Gotelli, 2012). The null model related to the function permatfull() from the R-package vegan (Oksanen et al., 2019), with both as permutation constraint, and using the count method for abundance data.

To test whether the FD_Q values significantly changed between groups of uncontaminated sites and contaminated sites, a one-way analyses of variances (ANOVA) followed by Tukey's HSD post-hoc test is used because parameter present homogenous variances according to the Leven's test ($Df = 2, F = 1.48, p = 0.26$) by using the aov () function in the R-package stats (R Core Team, 2020) and the leveneTest() function of the R-package car (Fox and Weisberg, 2019).

Finally, a hierarchical cluster analysis was applied to define functional groups (with similar combinations of traits) of macroinvertebrates in the functional space shaped by the first two axes of PCoA using the hclust() function of the R-package stats (R Core Team, 2020). The cluster analysis was based on Euclidean distance between the relative position of species in the functional space defined by the two first axes of PCoA. The Ward's minimum variance method was used for assembling taxa in successive larger groups (Ward, 1963). To determine whether trait composition differed significantly among functional groups, we performed a multi-response permutation procedure (MRPP), a non-parametric procedure, on the functional trait Euclidean distance matrix using the mrpp() function of the R-package vegan (Oksanen et al., 2019). The MRPP's test statistic ($A = 1.967, p < 0.0001$; Fig. S1) points out a significant functional dissimilarity among-functional groups, suggesting group-specific combinations of traits (Fig. S2), and a relatively high functional homogeneity within functional groups.

3. Results

3.1. Contrasted responses of taxonomic and functional diversities to regional metal contamination

Thanks to the integrated multi-substance indicator (IBC_{metals}) calculated from the database on the bioavailable contamination levels of more than 200 sites throughout France (Alric et al., 2019), we selected a set of 23 sites presenting a metal contamination gradient and distributed at a regional scale in the South West of France (Fig. 1A). The calculation of the species accumulation curve, based on all species ($n = 97$) recorded at all sites (Fig. S3) reveals a saturation pattern, pointing out that the number of sites selected in our study allows the identification of the almost totality of stream invertebrate species present in this freshwater environment at this regional scale. According to the Preston's lognormal model, we estimate that only three rare species among the expected species reservoir could have been unidentified during the field sampling of the 23 sites. The 90%-quantile regression of Shannon diversity index (or Hill number of order 1) against IBC_{metals} reveals a strong reduction in the upper level of taxonomic diversity along the gradient of metal contamination ($p = 0.017$; Fig. 1B). No significant relationship was found between the decreasing of Shannon diversity index and the other physico-chemical parameters (Table S4). This result indicated that the observed reduction of taxonomic diversity is not related to environmental parameters, known to influence diversity. Similar results were obtained when species richness was considered (Table S5).

According to the regional median value of Shannon diversity index ($Shannon\ diveristy\ index = 9.41$; Table S6), two subgroups among uncontaminated sites ($IBC_{metals} = 0, n = 10$, thereafter called "UnCont") can be distinguished. A first subgroup UnCont1 ($n = 4$) with higher taxonomic diversity than the regional median value ($Shannon\ diversity\ index > 10, mean = 11.9, sd = 1.9$), and a second subgroup UnCont2 ($n = 6$) with lower taxonomic diversity ($Shannon\ diveristy\ index < 7$, $mean = 5.4, sd = 1.0$). In addition, a group of sites ($n = 6$) were considered as contaminated sites (thereafter called "Cont") because they had metal contamination levels in the highest range of regional values (75%-quantile of regional $IBC_{metals} =$ 0.256; Table 1; Table S7). Sites in Cont group exhibited also values of Shannon diversity index lower than the regional median value. The observed reduction in the taxonomic diversity between uncontaminated sites having the higher taxonomic diversity (i.e., UnCont1) and contaminated sites results in an average loss of 14 species.

To test the concurrent effect of metal contamination on the functional diversity of macroinvertebrate assemblages, we focused on sites located at both ends of the contamination gradient, i.e., UnCont1, UnCont2, and Cont groups of sites. Contrary to the decreasing pattern in taxonomic diversity, no significant difference in functional diversity values (i.e., FD_Q) is detected between the three groups of sites ($Df = 2$, $F = 0.228, p = 0.799$; Fig. 1C). The PCoA representing the functional space occupied by the whole set of species of the study area also underpinned this similarity in macroinvertebrate functional diversity between contaminated and uncontaminated streams: species occurring in UnCont1 ($n = 83$) occupy a large volume in this functional space and overlap strongly with the species occurring in each of the two other groups of sites ($n = 82$ for UnCont2 and $n = 69$ for Cont) (Fig. S4).

3.2. Species replacement between uncontaminated and metal-contaminated streams

We then focused on the UntCon1 and Cont groups of sites to evaluate the processes explaining the divergence of species assemblages along the drop of taxonomic diversity associated with metal contamination. A significant deviation in the observed beta diversity from the null expectation is detected when both incidence and abundance data are used (incidence: $p = 0.0003$, abundance: $p = 0.0001$), revealing a non-random spatial structuring of macroinvertebrate assemblages related to metal contamination at the regional scale. Regarding the possible deterministic processes known to structure spatial variability, incidence-based indices showed that the pattern of overall beta diversity among UnCont1 and Cont group of sites ($\beta_{sor} = 0.54$) is mainly explained by spatial turnover (76% contribution) relative to the nestedness of species assemblages (24% contribution). In the case of abundance-based indices, the pattern of overall beta diversity ($\beta_{BC} = 0.74$) is mostly caused by the balanced variation in abundances (84% contribution) relative to the abundance gradients (16% contribution).

Furthermore, the pools of dominant species identified thanks to a taxon scarcity metric appear to be dissimilar between Uncont1 and Cont (Fig. 2). Although these two groups of sites share out 28 common dominant species (among 50 dominant species in UnCont1 and 41 in Cont), different dominant species are specific either to uncontaminated or contaminated sites ($n = 22$ species for UnCont1 and $n = 13$ species for Cont). These UnCont1- and Cont-specific species are either dominant in one group but not in the other, or occurred exclusively in one group of sites (Fig. 2). Besides, the PCoA analysis describing the distribution of species throughout their trait-based functional space (Fig. 3A) reveals that the 28 common dominant species are found in seven of the ten functional groups identified by a cluster analysis of biological traits of the whole species data set (Fig. S5; Table S3), tested by a MRPP analysis (see Materials and Methods and Fig. S1) and exhibiting specific trait combinations (Fig. S2). Twenty UnCont1-specific dominant species (among 22) co-occur with Cont-specific dominant species in eight of the ten functional groups, and the two remaining UnCont1-specific dominant species occur in one single functional group (FG3). Symmetrically, only one (among 13) Cont-specific dominant species is found in a functional group with no UnCont1-specific dominant species counterpart (FG7). Interestingly, UnCont1- and Cont-specific dominant species contributed at similar levels to functional richness (estimated by the contribution metric) and functional divergence (estimated by the originality metric) in their respective communities (Fig. 3B). Furthermore, the functional dissimilarity among UnCont1-specific dominant species is moderately lower than that among Cont-specific dominant species whatever the normalization procedure considered (e.g., UnCont1: $\beta = 1.15$; Cont:$\beta = 1.28$; see Table S8).

4. Discussion

Studying the ecological impact of micropollutant contamination into freshwater ecosystems is a societal concern (Bennett et al., 2015). Humans indeed depend, socially and economically, on the services that these ecosystems provide, including the production of food and the maintenance of hydrological cycles (Aylward et al., 2005; Grizzetti et al., 2016; Hanna et al., 2018). Recently, the need for a fruitful exchange of concepts and methods between ecologists and ecotoxicologists to improve our understanding of the effects of micropollutant contamination on biodiversity has been highlighted (Beketov and Liess, 2012; Gessner and Tlili, 2016). Therefore, taking advantage of the reliable assessment of exposure of communities to contamination offered by an active biomonitoring methodology (Alric et al., 2019) used in national monitoring programs implemented by French water agencies and by considering advanced concepts from community ecology, we investigated at a regional spatial scale how bioavailable metal contamination affects the integrity of stream macroinvertebrate communities. Based on 23 study sites presenting a gradient of metal contamination, comparable environmental conditions (altitude, size, hydroecoregion, absence of POPs contamination), and harbouring a representative sample of the regional pool of species, our study extends to the structural level of communities (i.e., the alpha diversity) previous results, which already demonstrated the impact of bioavailable metal contamination on the abundance of few specific macroinvertebrate species (e.g., Luoma et al., 2010; Schmidt et al., 2011; Alric et al., 2019). The present observation of a decline in the taxonomic diversity of macroinvertebrates in French watercourses confirms the deleterious effect on regional freshwater biodiversity of metal contamination (Clements et al., 2000), as demonstrated for pesticides (Beketov et al., 2013) and more generally for other agents of global change (Peirera et al., 2012; Foley et al., 2005). We paid attention to potential confounding factors in our analysis, and no significant effects of other environmental parameters on the taxonomic diversity of macroinvertebrate communities were detected among the 23 investigated streams. Nevertheless, our assessment of non-metallic contamination is based on bioaccumulation data for 43 POP compounds (including priority substances of the WFD; Alric et al., 2019). We have to point that the vast majority of pesticides used in agriculture are not covered by this list of monitored substances, even if some pesticides were considered (4,4'-DDD, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, dicofol, HCH γ (lindane), pendimethalin). So, we cannot fully rule out the hypothesis of a confounding effect of pesticides could occur in our study region, given that pesticides can have a non-negligible effect on structure of macroinvertebrate communities (Beketov et al., 2013).

Contrary to the expectation of a congruent pattern between the two facets of biodiversity (Bhin et al., 2010; Pool et al., 2014), the negative trend for taxonomic diversity does not translate into similar trend for functional diversity (Fig. 1). This trend follows previous works reporting that functional diversity may fluctuates quite independently of taxonomic diversity in terrestrial communities (Cadotte et al., 2011; Carmona et al., 2012) as in aquatic communities (e.g., macroinvertebrates; Reynaga et al., 2013; Voß and Schäfer, 2017). Furthermore, similar to our results, two previous works had also shown in their study sites that functional diversity was marginally associated (Liess et al., 2021) or not (Voß and Schäfer, 2017) with environmental pressures. It has been argued that the type of traits used for quantifying functional diversity, the choice of functional diversity metrics, the level of functional redundancy, the size of taxon pool and the strength of environmental filters can all shape the relationships between these two facets of biodiversity (Cadotte et al., 2011). To estimate functional diversity, we used the Rao's quadratic entropy considered as robust to highlight differences in functional diversity in response to environmental pressures (Brown and Milner, 2012; Gusmao et al., 2016). Similar results were obtained with the functional dispersion index proposed by Laliberté and Legendre (2010) (Fig. S6 and Fig. S7; see also Material S2). We also used the recommended type of functional traits (i.e., biological traits) reflecting the effect of organisms on ecosystem functions (Violle et al., 2007), that are known to be sensitive to human-induced pressures (Statzner and Bêche, 2010; Lange et al., 2014), including micropollutant pollution (Archaimbault et al., 2010; Pallotini et al., 2016). While, a small size of taxon pool has been highlighted as maying restrict a strong functional response to environmental pressures (Voß and Schäfer, 2017), our study sites harboured a representative sample of the regional pool of species (Fig. S3). An alternative reason for inconsistencies between the expected decreasing of functional diversity and our field experimental result could be the insufficient amplitude of the disturbance gradient (Gerisch et al., 2012), which may have been too short to influence functional diversity. Nevertheless, the gradient of metal contamination studied in the selected region ($0 \leq IBC_{metals} \leq 0.511$) fully covers the maximal range of contamination levels observed at the French national scale (95%-quantile of national $IBC_{metals} = 0.482$; Alric et al., 2019). This ensures the environmental relevance of the findings of this regional pattern.

Different ecological mechanisms may explain the non-response of functional diversity to the bioavailable metal contamination, in comparison with taxonomic diversity reduction. Flynn et al. (2009) suggested that following a decrease in taxonomic diversity in response to environmental degradation (e.g., land use intensification), functional diversity may remain constant by a non-random mechanism corresponding to the loss of functionally redundant species firstly. Behind this non-random mechanism, there is the idea that resulting assemblages at impacted sites are subsets of the assemblages encountered at unimpacted sites (Larsen and Ormerod, 2010). Rather than supporting this nestedness pattern of species assemblages, our results show in agreement with a recent meta-analysis (Soininen et al., 2018) that taxonomic-based beta diversity was mainly driven by a non-random mechanism of spatial turnover. The spatial turnover is here expressed not only as a shift in species dominance patterns along the contamination gradient (in term of abundance, β_{BC}) but also as a replacement of some species (β_{sor}). From a functional point of view, our analysis demonstrates that the taxonomic turnover along the metal contamination gradient is coupled with a compensation in the functional role (i.e., contribution to functional richness and functional divergence) of dominant species (Fig. 3) considered as typical drivers of ecosystem processes (Moore, 2006). Together these findings support the hypothesis that the loss of functional diversity following the loss of sensitive species due to the increase in level of an environmental disturbance (here bioavailable metal contamination) is balanced with the non-random replacement of species by functionally similar species (Bellwood et al., 2006). Our results confirm those of a previous work, which assumed that the lack of effect of pesticides on functional diversity observed in their study sites was probably due to compensatory processes, where the loss of sensitive species can be compensated by tolerant species (Liess et al., 2021).

Our empirical work provides evidence of a dynamic response of regional macroinvertebrate community to change in bioavailable metal contamination levels with a decoupling between taxonomic and functional diversity. We documented a loss of species but a maintenance of functional diversity relying on a process of non-random species replacement by functional redundant species in contaminated streams. Our results have important implications at the ecosystem level, as the maintenance of functional diversity after species replacement can go hand in hand with the maintenance of ecosystem functions (Little and Altermatt, 2018). Such decoupling underpinning the stability of functional redundancy may strengthen the ability of macroinvertebrate communities to withstand environmental change. Nevertheless, metal-tolerant species would be expected to exhibit specific adaptations to metal toxic stress, which can go with physiological costs or specific trade-offs between life history traits reducing their ability to cope with other environmental disturbances (see Vigneron et al. (2015) for an example of reduced feeding activity in a cadmium-tolerant population of the freshwater shredder Gammarus). Considering such potential fitness costs of metal-tolerance, one remaining question is therefore how far the observed functional resilience based on the selection of peculiar metal-tolerant species among the regional pool of species is still operant in multi-stressed aquatic environments.

5. Conclusion

This study provides new insights into the consequences of bioavailable metal contamination on the integrity of freshwater macroinvertebrate communities. While an expected degradation in structural integrity of communities was observed in response to increased bioavailable metal contamination, no significant changes were observed in their functional integrity. Analysis of regional community structure supports the hypothesis that niche-selection filtering rather than stochastic determines the functional integrity of macroinvertebrate communities along the gradient of bioavailable metal contamination, relying on a non-random process of species replacement by functional redundant species. Such findings call for questioning the vulnerability of metal-tolerant communities in multi-stressed environments. The underlying rationale is to test how metal-tolerant species (i.e., Cont-specific dominant species) cope with, for example, other types of pressures of human origin such as temperature stress, contamination by

POPs or pesticides, to assess whether functional diversity is maintained in multi-stress contexts and to estimate consequences on ecosystem functions.

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Author statement

Benjamin Alric: Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Supervision, Writing – Original Draft, Writing – Review & Editing; Olivier Geffard: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – Review & Editing; Arnaud Chaumot: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – Review & Editing.

Competing Interests

The achievement of this manuscript has not been influenced by competing financial, personal, or professional interests.

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Figures

Fig. 1. Taxonomic and functional diversity along the metal contamination gradient. (A) Map of the study watershed (Adour-Garonne, South West of France) with the geographic location of sampling sites (dots) into the hydrographic network. In A, the insert corresponds to the map representing the geographic delimitation of boundaries of six French watersheds. (B) Shannon diversity index as a function of the metal contamination (expressed from IBC_{metals}). Black solid line indicates the regression fitted at 90th percentile distribution. Based on the AIC value, the linear model ($AIC = 122.525$) was the best model to fit the tested relationships in relation to curvilinear model ($AIC = 122.632$). The dots with a blue circle correspond to sites belong to the first uncontaminated ($IBC_{metals} = 0$) group of site (i.e., UnCont1) characterized by a high taxonomic diversity ($Shannondiversityindex > 10$), whereas dots circled in green belong to the second uncontaminated group of sites (i.e., UnCont2) characterized by lower taxonomic diversity ($Shannon\ diversity\ index < 7$), and dots circled in red belong to the contaminated ($IBC_{metals} > 0.26$) group of sites (i.e., Cont). (C) Rao's quadratic entropy (FD_Q) estimated in uncontaminated (UnCont1 and UnCont2) and contaminated (Cont) groups of sites. Colored dots represent Rao's quadratic entropy for each sampling sites, whereas black triangles represent the average Rao's quadratic entropy in each group of sites. Letters above each group of sites indicate significant difference based on one-way analyses of variance (ANOVA) followed by Tukey's HSD post-hoc test.

Fig. 2. Species sorted according to their average values of scarcity, A) in group of uncontaminated sites (UnCont1) and B) in group of contaminated sites (Cont). To simplify the interpretation, we used $1 - scarcity$, meaning that $1 - scarcity$ gets close

to one when the species dominates the assemblages and gets close to zero when the species has low abundance. In each panel (A and B), species above the dashed horizontal line had a $1 - scarcity$ higher than the 40th percentile and they are considered as dominant species. Labels of these species are colored according to their occurrence in the two groups of sites. Dominant species occurring specifically in UnCont1 are in blue (panel A), whereas dominant species occurring specifically in Cont are in red (panel B), and dominant species occurring in both groups are in black. In panel A, gold triangles report the scarcity valuse of the dominant species in Cont occurring also in UnCont1. Symbols * identified dominant species occurring exclusively in one group of sites, and symbols ** identify species dominant in one group of sites but not dominant in the other group.

Fig. 3. Species replacement and functional contribution between uncontaminated and metal-contaminated streams. (A) Principal Coordinates Analysis (PCoA) representing the functional space of macroinvertebrates based on their functional traits. The two axes represent the two synthetic functional dimensions obtained from PCoA based on the functional distance matrix (Euclidean distance). Each species is represented by a dot colored: dominant species occurring exclusively in the uncontaminated group of sites (UnCont1) in blue, dominant species occurring exclusively in the contaminated group of sites (Cont) in red, dominant species occurring in both groups of sites (Common) in dark grey, and rare species (Rare) in light grey. Convex hulls define groups of species with different trait profiles. These functional groups (FG) were identified using a hierarchical clustering analysis on the dissimilarity matrix (Euclidean distance) in the position of species into the functional space shaped by the first two axes of PCoCA. See Table S2 for the list of species and their memberships to the different functional groups. (B)

Functional contribution and functional originality for dominant species common (dark grey) to uncontaminated (UnCont1) and contaminated (Cont) sites and for dominant species specific (Uncont1: blue, Cont: red) to one of the two group of sites.

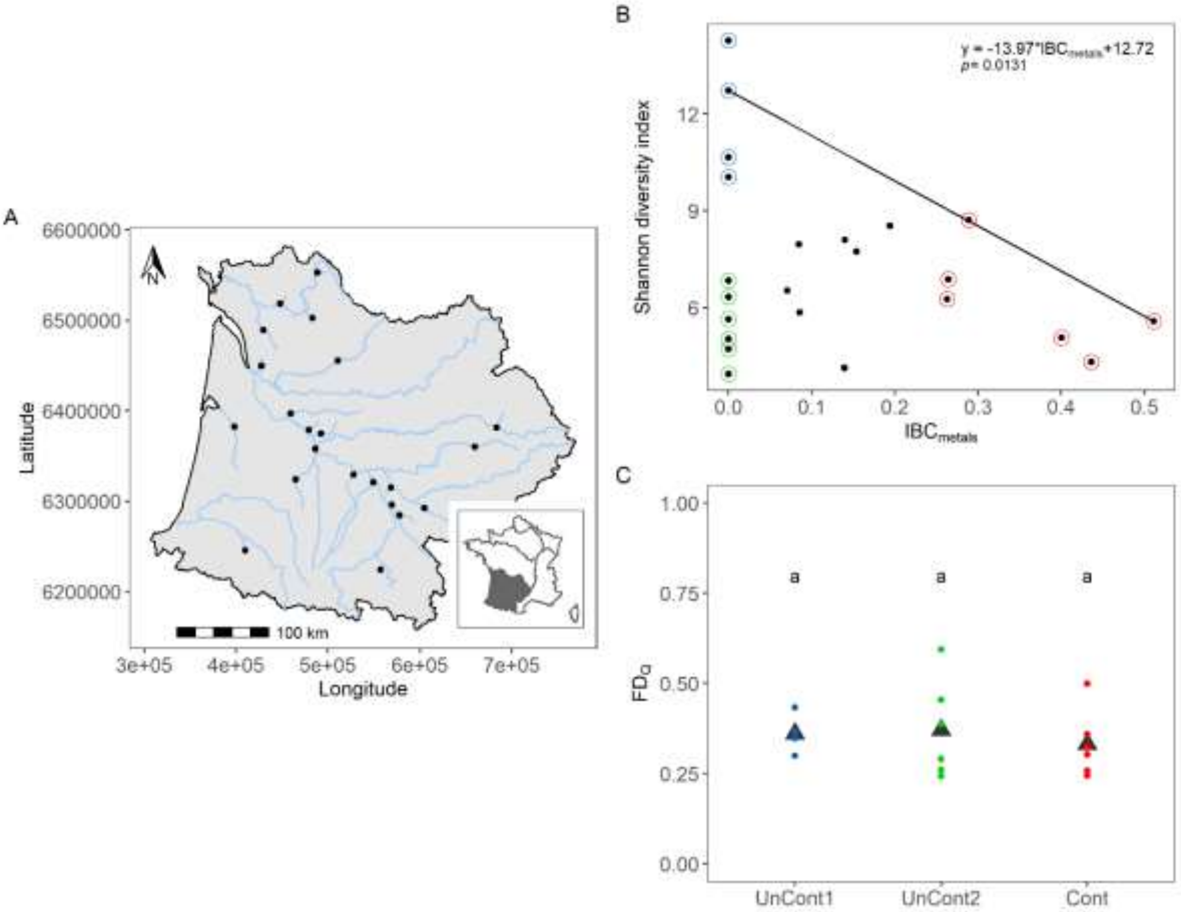

A
6600000
6500000
6400000
6300000
6200000
Latitude
N
100 km
3e+05
4e+05
5e+05
6e+05
7e+05
Longitude
B
y = -13.97*IBC_metals+12.72
p= 0.0131
Shannon diversity index
12
9
6
0.0
0.1
0.2
0.3
0.4
0.5
IBC_metals
C
1.00
0.75
0.50
0.25
0.00
FD_Q
a
a
a
UnCont1
UnCont2
Cont

Figure 1.

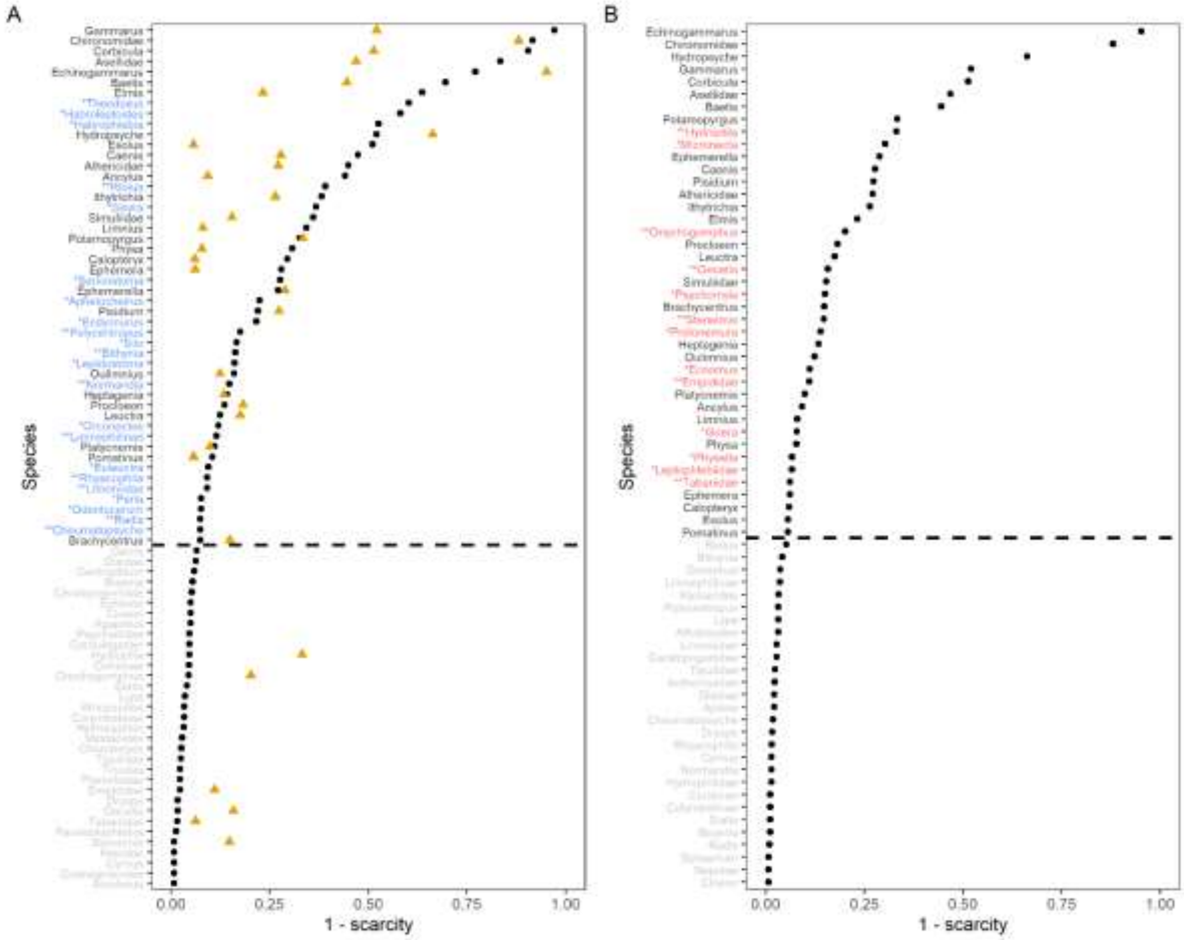

A
B
Species
1 - scarcity
0.00
0.25
0.50
0.75
1.00
Gammarus
Chironomidae
Corbicula
Asellidae
Echinogammarus
Baetis
Elmis
*Theodoxus
*Habroleptoides
*Habrophlebia
Hydropsyche
Esolus
Caenis
Athericidae
Ancylus
Ithytrichia
Simuliidae
Limnius
Potamopyrgus
Physa
Calopteryx
Ephemera
Ephemerella
Pisidium
*Silo
*Lepidostoma
Oulimnius
**Normandia
Heptagenia
Procloeon
Leuctra
Platycnemis
Pomatinus
*Perla
*Odontocerum
**Cheumatopsyche
Brachycentrus
Echinogammarus
Chironomidae
Hydropsyche
Gammarus
Corbicula
Asellidae
Baetis
Potamopyrgus
*Micronecta
Ephemerella
Caenis
Pisidium
Athericidae
Ithytrichia
Elmis
**Onychogomphus
Procloeon
Leuctra
Simuliidae
*Psychomyia
Brachycentrus
*Protonemura
Heptagenia
Oulimnius
*Ecnomus
**Empididae
Platycnemis
Ancylus
Limnius
Physa
*Physella
*Leptophlebiidae
**Tabanidae
Ephemera
Calopteryx
Esolus
Pomatinus

Figure 2.

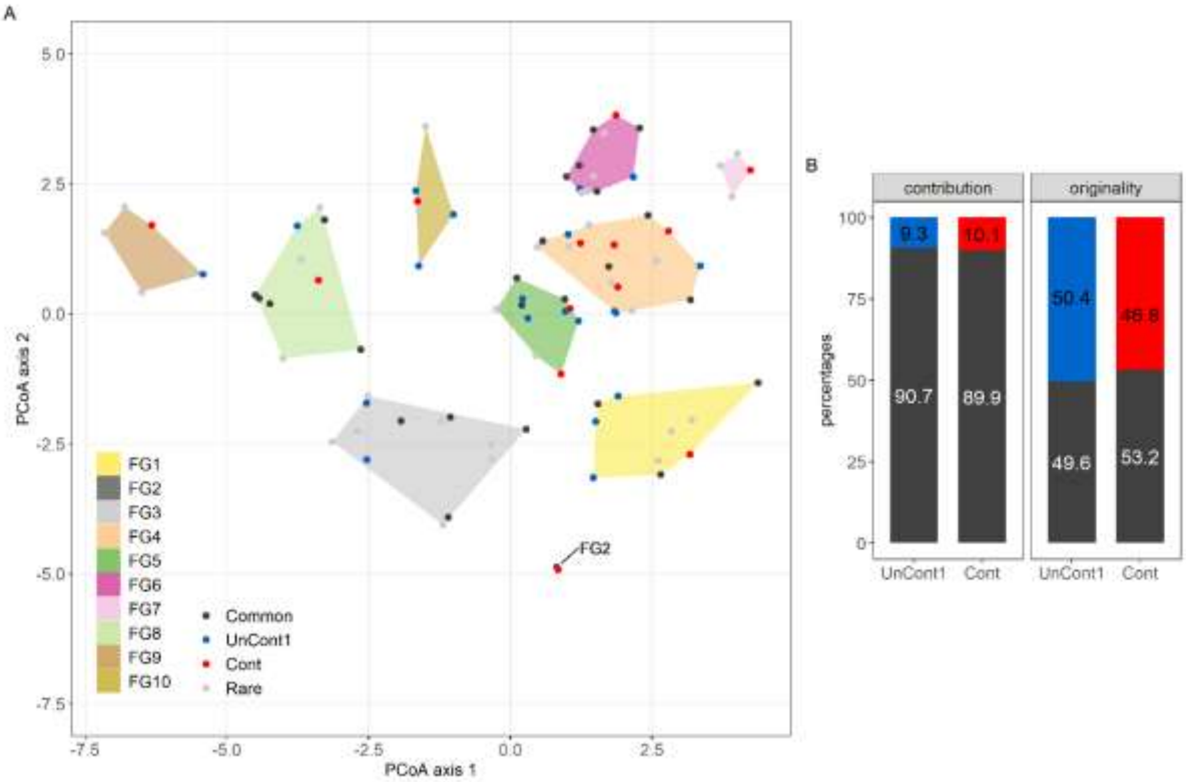
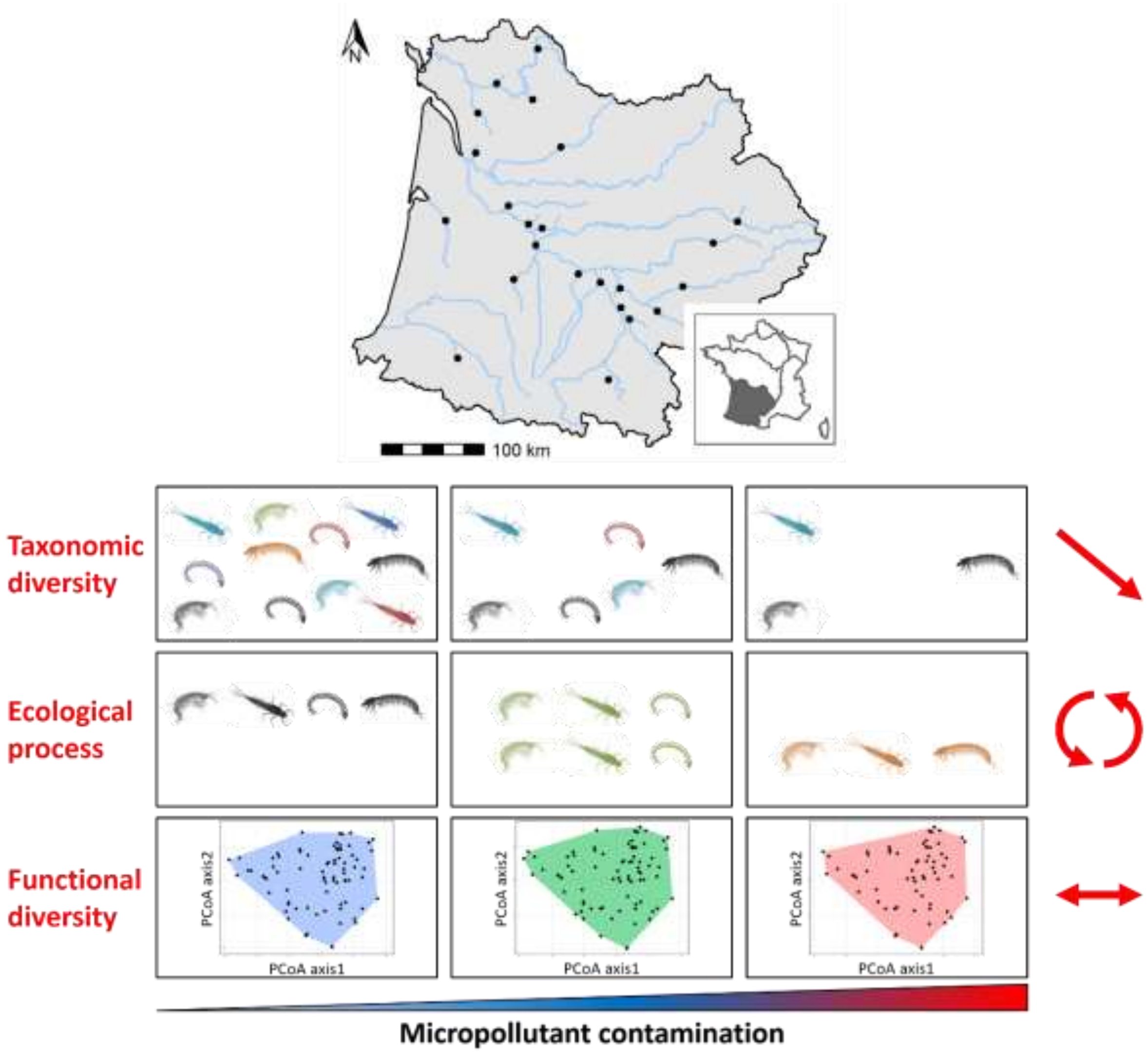

A
PCoA axis 2
PCoA axis 1
5.0
2.5
0.0
-2.5
-5.0
-7.5
-7.5
-5.0
-2.5
0.0
2.5
FG2
FG1
FG2
FG3
FG4
FG5
FG6
FG7
FG8
FG9
FG10
Common
UnCont1
Cont
Rare
B
contribution
originality
percentages
100
75
50
25
0
9.3
10.1
90.7
89.9
50.4
46.8
49.6
53.2
UnCont1
Cont
UnCont1
Cont

Figure 3.

Table 1. Contextual characteristics of 23 sites (id) used in the study. The x and y variables are the geographic coordinates estimated in Lambert93 projection system. Elevation (altitude) is in meters. The strahler variable is the Strahler's stream order, a positive integer used in geomorphology and hydrology to indicate the level of branching in a river system. Hydroecoregion is a typology of streams considering a climatic, hydrogeological, and landform hydroecological classification (Wasson et al. 2002).1: Pyrénées (high mountain), 3: Massif Central Sud (mountain), 9: Tables Calcaires (plain), 11: Causses Aquitains (small relief), 13: Landes (plain), 14: Côteaux Aquitains (small relief). IBC_{metals} is the bioavailable metal contamination level in each stream estimated from the multi-substance indicator (Alric et al., 2019). In addition to the parameters presented in the table, 20 other physico-chemical parameters were measured: BDO5, Ca, Chl-a, Cl, Conductivity, Hydrometric Title (HT), K, Mg, Na, NH_4^+, NO_2^-, NO_3^-, O_2, pH, Pheopigment, PO_4^{3-}, POC, SiO_4^{2-}, temperature, Total Suspended Solids (TSS).

id	x	y	altitude	strahler	hydroecoregion	IBC_{metals}
5023100	488325	6553063	98	1	9	0.0000
5039000	510935	6455395	76	4	14	0.0000
5176100	557216	6224434	322	2	1	0.0000
5211550	409969	6245768	214	1	14	0.0000
5013150	447895	6518717	13	1	9	0.0000
5015320	483036	6502550	105	1	11	0.0000
5025830	427349	6449524	9	3	14	0.0000
5079100	459459	6397156	17	4	14	0.0000
5134000	605082	6292402	117	5	14	0.0000

5153000	549220	6321073	82	5	14	0.0000
5099170	683487	6381272	392	3	3	0.0705
5008000	429785	6489387	34	1	9	0.0844
5118000	527866	6329248	67	4	14	0.0855
5129150	565822	6315269	90	2	14	0.1395
5161000	486212	6358047	117	5	14	0.1398
5127000	659597	6360008	493	4	3	0.1536
5142100	629764	6316922	139	4	3	0.1939
5191900	398433	6381859	24	5	13	0.2627
5083585	492143	6374725	45	3	14	0.2643
5156950	569551	6295940	116	5	14	0.2889
5083300	479204	6378802	30	4	14	0.4004
5106850	464841	6323918	94	3	14	0.4361
5157100	577655	6284259	136	3	14	0.5112


N
100 km
Taxonomic diversity
Ecological process
Functional diversity
PCoA axis2
PCoA axis1
PCoA axis2
PCoA axis1
PCoA axis2
PCoA axis1
Micropollutant contamination