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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10   Abstract

11   Freshwater ecosystems are the main source of water for sustaining life on earth, and the

12   biodiversity they support is the main source of valuable goods and services for human

13   populations. Despite growing recognition of the impairment of freshwater ecosystems

14   by micropollutant contamination, different conceptual and methodological

15   considerations can newly be addressed to improve our understanding of the ecological

16   impact into these ecosystems. Here, we originally combined in situ ecotoxicology and

17   community ecology concepts to unveil the mechanisms structuring macroinvertebrate

18   communities along a regional contamination gradient. For this, our study benefited from

19   the recent deployment of an innovative biomonitoring approach relying on the

20   measurement of metal bioaccumulation in caged crustaceans throughout the French

21   river monitoring network. We were hence able to identify 23 streams presenting a

22   significant gradient of bioavailable metal contamination within the same catchment area

23   in the South West of France, from which we also obtained data on the composition of

24   resident macroinvertebrate communities. Analyses of structural and functional integrity

25   of communities revealed an unexpected decoupling between taxonomic and functional

26   diversity of communities in response to bioavailable metal contamination. We show that

27   despite the negative impact of bioavailable metal contamination exposure on taxonomic

28   diversity (with an average species loss of 17% in contaminated streams), functional

29   diversity is maintained through a process of non-random species replacement by

30   functional redundant species at the regional scale. Such unanticipated findings call for a

31   deeper characterization of metal-tolerant communities' ability to cope with

32   environmental variability in multi-stressed ecosystems.

33   Keywords

34   freshwater macroinvertebrate, taxonomic diversity, functional diversity, functional

35   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 CO_2 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).

60   Beyond these shifts in taxonomic composition (structural component),

61   community integrity also has a functional component, and both must be considered

62   simultaneously when assessing the impairment of macroinvertebrate communities

63   (Minshall 1996). Functional integrity can be assessed via the measure of functional

64   diversity from a multiple-trait-based approach (Statzner at al., 2001). The rationale for

65   the complementary measure of functional diversity is that it provides clearer

66   mechanistic links to ecosystem services, since species traits are the main properties by

67   which organisms influence ecosystem processes (Petchey and Gaston, 2006; Gagic et al.,

68   2015). In the simplest scenario, changes in both taxonomic and functional diversity are

69   congruent (Brice et al., 2017; Jarzyna and Jetz, 2017). But they can also diverge from this

70   expectation with a decoupling between both (Purschke et al., 2013; Robroek et al., 2017)

71   as a result of high functional distinctness between species (Sonnier et al., 2014; White et

72   al., 2018) or high functional similarity (i.e., functional redundancy; Villéger et al., 2014;

73   Mori et al., 2015). The evaluation of these alternative co-variation patterns between

74   functional and taxonomic diversity is essential to predict the future ecological

75   trajectories of ecosystems in response to anthropogenic pressures including

76   micropollutant contamination.

77   On a more methodological point of view, the level of metal exposure of aquatic

78   communities is usually estimated from chemical measurements in the water column

79   and/or sediments in the majority of field studies. Detecting chemicals in these

80   environmental compartments does not integrate the bioavailability of contaminants,

81   which conditions harmful effects on organisms. That is why the European Water

82   Framework Directive (WFD) increasingly integrates contaminant measurements in

83   biota (Wernersson et al., 2015). This approach displays several advantages: (i) the

84   bioconcentration of contaminants by organisms leads to easier and accurate

85 quantifications of micropollutants; (ii) these measurements reflect the bioavailable

86 fraction (potentially toxic); (iii) they constitute time-integrated measurements (Besse et

87 al., 2012; Inostroza et al., 2016). Measurements in biota can be applied in passive

88 biomonitoring approach, using indigenous organisms (e.g., Kraak et al., 1991; Caçador et

89 al., 2012; Bertrand et al., 2018), or in active biomonitoring approach, using field-caged

90 organisms originated from natural or farmed reference populations (e.g., Mersch et al.,

91 1996; Oikari, 2006; Prygiel et al., 2016). Active biomonitoring approach is based on

92 "standardized" organisms (gender, size, physiological status) from a reference

93 population, allowing like this to limit the impact of biological confounding factors known

94 to modulate accumulation (Besse et al., 2012). Moreover, this approach allows to control

95 exposure time (history), providing a reliable comparison of measured bioavailable

96 contamination levels in space and time (between sampling events; Burton et al., 2005;

97 Shahid et al., 2018). The caging methodology proposed by Besse et al. (2013) with the

98 sentinel species Gammarus fossarum has been recently adapted and standardized by the

99 French agency AFNOR (AFNOR, 2019) and then was used by French water agencies for

100 the national chemical survey of waterbodies. Over the period from 2009 to 2016, this

101 biomonitoring tool has been deployed at more than 200 stations (Alric et al., 2019)

102 belonging to two major monitoring networks established by French water agencies

103 implementing the WFD requirements, the surveillance monitoring network and the

104 operational monitoring network, on which 18 metals and 43 persistent organics

105 pollutants (POPs) have been researched, including priority substances of the WFD

106 (European Commission, 2008). From this database of French water agencies, Alric et al.

107 (2019) developed multi-substance indicators (IBC) to estimate the overall

108 contamination level in aquatic ecosystems. One indicator was developed for metals

109 (IBC_{metals}) and another for POPs (IBC_{POPs}). The strength of such an indicator lies in the

110   fact that it represents the bioavailable contamination fraction, is integrative and allows a

111   reliable comparison of contamination levels in space and time, and thus offers the

112   opportunity to question/study the community response to environmental

113   contamination. In addition, Alric et al. (2019) observed a relationship between

114   bioavailable metal contamination and reduction of abundance, at the national scale, of a

115   few sensitive macroinvertebrate families.

116   The aim of this study is to investigate alterations in the integrity (i.e., structural

117   and functional) of macroinvertebrate communities in response to bioavailable metal

118   contamination at a regional scale. This purpose was possible by using the database built

119   by the French water agencies through the national chemical biomonitoring survey of

120   waterbodies with caged G. fossarum and previously exploited and presented by Alric et

121   al. (2019). Our goal was (i) to evaluate detailed changes in both taxonomic and

122   functional diversity in response to a metal contamination gradient, (ii) to examine the

123   congruence or divergence of these changes and (iii) to identify underlying ecological

124   processes. Hence, this regional study made it possible to understand changes in the

125   composition of communities by testing the hypotheses of erosion (i.e., simple loss with

126   selection of resistant) vs species replacement and to interpret the modifications of local

127   species assemblages in terms of functions potentially realized in stream ecosystems.

128   **2.** Materials and Methods

129   2.1.   Datasets

130   To investigate the impact of metal chemical pressure on the integrity (structural and

131   functional) of stream macroinvertebrate we used data extracted from the database of

132   the national chemical survey conducted by the French water agencies and presented by

133   Alric et al. (2019). The objective here was to have sites exhibiting similar contextual

134 characteristics (watershed, size range, elevation range) and showing a gradient of

135 bioavailable metal contamination. Twenty-three sites meeting these requirements were

136 selected, located in the Adour-Garonne catchment (the South West of France) which

137 drains an area of 117,650 km2. These sites have comparable environmental settings

138 with stream elevations ranging from 9 to 493 m, stream sizes (stream order) according

139 to the Strahler number ranging from 1 to 5 and streams locations (identity of the

140 hydroecoregion according to Wasson et al., 2002) mainly in lowland and small relief

141 (Table 1). For each sampling site, 20 physico-chemical parameters (Table 1; Table S1)

142 were available and will be used in order to subsequently test for potential confounding

143 effects on the structural integrity of macroinvertebrate communities. These sites also

144 exhibited a gradient of metal contamination (Table 1) and were free for POPs followed

145 by the French water agencies in the biota support, namely PAHs, PCBs, and

146 organochlorine pesticides. Given that study sites are subject to temporal survey of

147 macroinvertebrates as part of the monitoring programs implemented in France by the

148 WFD, the information on macroinvertebrates communities (abundance of taxa) was

149 extracted from the national database "naiades" (http://www.naiades.eaufrance.fr)

150 according to a temporal matching with dates of caging. Standardized protocols,

151 following the recommendations of national and international standards, are used to

152 sample macroinvertebrates annually between May and October (multi-habitat sampling

153 protocol; AFNOR, 2009) and to characterize benthic macroinvertebrate assemblages

154 (AFNOR, 2010; Table S2).

155 2.2.   Metrics of taxonomic diversity

156 A species accumulation curve was calculated to estimate whether the sampling effort

157 realized in the considered regional area (i.e., the spatial extent covered by the 23 sites)

158 was sufficient to collect most of the species occurring. The accumulation curve was

159 calculated from 9,999 random permutations of all the sampling sites constituting our

160 dataset without replacement (Gotelli and Colwell, 2001) and by using the jackknife

161 estimator (here of the order 1) to estimate the species richness. The jackknife estimator

162 presents the advantage to provide reliable estimation of richness even though the

163 sampling effort is low (Brose et al., 2006). The accumulation of local diversity, described

164 by the species accumulation curve, allows to infer the sample completeness for a given

165 number of investigated sites. The structural diversity within each sampling site (i.e., the

166 alpha diversity) was calculated using the Shannon diversity index based on the Shannon

167 entropy with a logarithm to the base 2 (Material S1). The Hill number of order 1,

168 corresponding to the exponential of the Shannon entropy with a natural logarithm (Jost,

169 2006), was also calculated (Material S1) as well as the species richness.

170   The overall beta diversity, which refers to site-to-site composition dissimilarity,

171 was estimated with the Sørensen dissimilarity index based on incidence data (β_{sor}) and

172 partitioned into two components following the method proposed by Baselga (2010) to

173 assess if beta diversity patterns were structured more through spatial turnover (β_{sim}) or

174 nestedness of species assemblages (β_{nes}) (two patterns relying on deterministic

175 processes). A typical spatial species turnover pattern occurs when species at one site are

176 replaced by different species at another site. Nestedness occurs when the species pool

177 present in one site is a subset of that of another more species-rich site. The

178 characterization of the overall beta diversity was also realized by computing the Bray-

179 Curtis index of abundance-based dissimilarity (β_{BC}) and partitioning it into two

180 components, either balanced variation in species abundances (β_{BC-bal}) or abundance

181 gradients (β_{BC-gra}) (Baselga, 2013). Balanced variation in abundances occurs when the

182 individuals of some species in one site are substituted by the same number of

183   individuals of different species in another site, whereas abundance gradients occur

184   when the number of individuals of all species change from one site to the other with the

185   same sign. In the same way that β_{BC} reduces to β_{sor} when incidence data are used,

186   β_{BC-bal} and β_{BC-gra} are analogous to β_{sim} and β_{nes} respectively (Baselga, 2013).

187   Metrics were computed in the R software (R Core Team, 2020) using the R-

188   package vegan (Oksanen et al., 2019) whom the specaccum() function to compute the

189   species accumulation curve, the prestonfit() and veiledspec() functions to infer the

190   species richness, and the diversity() function to estimate the Shannon entropy. The

191   beta.pair() and beta.pair.abund() functions in the R-package betapart (Baselga et al.,

192   2018) were used to compute the overall beta diversity and partition it from the

193   incidence and abundance data respectively.

194   2.3.   Metrics of functional structure

195   Functional diversity of each macroinvertebrate assemblage was based on a species $\times$

196   traits matrix compiled from Usseglio-Polatera et al. (2000) and Tachet et al. (2010).

197   Taxa were characterized by 60 categories of eleven biological traits (Table S3), which

198   were found to be sensitive to micropollutant contamination (Archaimbault et al., 2010).

199   To translate available autoecological information about selected traits into numerical

200   values, a score was assigned to each taxon describing its affinity for that category using a

201   fuzzy-coding technique (Chevenet et al., 1994). Scores range from 0 to 5 indicating no

202   affinity and high affinity of the taxon for a given trait modality, respectively.

203   Based on the species $\times$ traits matrix and the relative abundance of each species,

204   the Rao's quadratic entropy (FD_Q) was computed to estimate the functional diversity of

205   macroinvertebrates assemblages using the modified formula proposed by Champely and

206   Chessel (2002) (Material S2). Given that each trait modality exhibited continuous values,

207 the functional distances matrix between each pair of species used in FD_Q was computed

208 using Euclidean distance. The rational choice to use FD_Q is provided in Material S2. To

209 visualize the relative position of macroinvertebrate species in the functional space, a

210 Principal Coordinates Analysis (PCoA) was then carried out on the functional distance

211 matrix (Euclidean distance) using the pcoa() function of the R-package ape (Paradis and

212 Schielp, 2018). The first two axes were selected as their explained variance (PC1: 12%

213 and PC2: 9%) was greater than the null expectation of a broken stick model (Legendre

214 and Legendre, 2012).

215   To propose a comprehensive analysis of the response of macroinvertebrate

216 assemblages to metal contamination, we focused on dominant species (in term of

217 abundance) because they are typically drivers of ecosystem processes (Moore, 2006).

218 Dominant species are defined as species having values of $1 - scarcity$ (Material S3)

219 higher than the 40%-quantile of the entire species pool of interest using the scarcity()

220 function of the R-package funrar (Grenié et al., 2017). Each species contributes

221 differently to the trait space occupied by their community. Different metrics, including

222 contribution and originality (Material S3), have been developed to quantify how

223 dissimilar is a species from all others within a community and hence how much it adds

224 to the trait space occupied by the community. We therefore estimated the contribution

225 and originality of dominant species using the eponymous functions in the R-package

226 BAT (Cardoso et al., 2021). We also estimated beta functional diversity among dominant

227 species by decomposing diversity according to the Rao's apportionment quadratic

228 entropy framework (Pavoine et al., 2016) using the EqRS() function of the R-package

229 adiv (Pavoine, 2020), with eq, normed1 or normed2 as consecutive normalizations.

230 2.4.   Data analysis

231   To test whether alpha diversity and metal contamination were associated, the Shannon

232   diversity values (Hill number of order 1 and species richness) were modelled as a

233   function of the contamination levels (expressed as the values of IBC_{metals}) using a

234   quantile regression analyses and the R-package quantreg (Koenker, 2018). Quantile

235   regression coefficients were estimated for percentile (τ) equal to 90%. Linear and

236   curvilinear (exponential) models were built and selection procedure based on Akaike

237   Information Criteria (AIC) was used to determine which model (linear or curvilinear)

238   best fits the data.

239   Following a null model approach, we tested whether the observed value of

240   overall beta diversity (i.e., β_{BC}, β_{sor}) differed from the value resulting to random species

241   assembly. The null distribution of beta diversity was estimated by generating 9,999

242   random assemblage matrices according to the fixed-fixed (FF) null model (Material S4).

243   Depending on the trade-off of not being too prone to both Type I (rejecting a true null

244   hypothesis) and Type II (failing to reject a false null hypothesis) statistical errors, this

245   null model is fairly robust compared to other null models (Gotelli and Ulrich, 2012;

246   Ulrich and Gotelli, 2012). The null model related to the function permatfull() from the R-

247   package vegan (Oksanen et al., 2019), with both as permutation constraint, and using

248   the count method for abundance data.

249   To test whether the FD_Q values significantly changed between groups of

250   uncontaminated sites and contaminated sites, a one-way analyses of variances (ANOVA)

251   followed by Tukey's HSD post-hoc test is used because parameter present homogenous

252   variances according to the Leven's test ($Df = 2, F = 1.48, p = 0.26$) by using the aov ()

253   function in the R-package stats (R Core Team, 2020) and the leveneTest() function of the

254   R-package car (Fox and Weisberg, 2019).

255   Finally, a hierarchical cluster analysis was applied to define functional groups

256   (with similar combinations of traits) of macroinvertebrates in the functional space

257   shaped by the first two axes of PCoA using the hclust() function of the R-package stats (R

258   Core Team, 2020). The cluster analysis was based on Euclidean distance between the

259   relative position of species in the functional space defined by the two first axes of PCoA.

260   The Ward's minimum variance method was used for assembling taxa in successive

261   larger groups (Ward, 1963). To determine whether trait composition differed

262   significantly among functional groups, we performed a multi-response permutation

263   procedure (MRPP), a non-parametric procedure, on the functional trait Euclidean

264   distance matrix using the mrpp() function of the R-package vegan (Oksanen et al., 2019).

265   The MRPP's test statistic ($A = 1.967, p < 0.0001$; Fig. S1) points out a significant

266   functional dissimilarity among-functional groups, suggesting group-specific

267   combinations of traits (Fig. S2), and a relatively high functional homogeneity within

268   functional groups.

269   **3.** Results

270   3.1.   Contrasted responses of taxonomic and functional diversities to regional metal

271   contamination

272   Thanks to the integrated multi-substance indicator (IBC_{metals}) calculated from the

273   database on the bioavailable contamination levels of more than 200 sites throughout

274   France (Alric et al., 2019), we selected a set of 23 sites presenting a metal contamination

275   gradient and distributed at a regional scale in the South West of France (Fig. 1A). The

276   calculation of the species accumulation curve, based on all species ($n = 97$) recorded at

277   all sites (Fig. S3) reveals a saturation pattern, pointing out that the number of sites

278   selected in our study allows the identification of the almost totality of stream

279   invertebrate species present in this freshwater environment at this regional scale.

280   According to the Preston's lognormal model, we estimate that only three rare species

281   among the expected species reservoir could have been unidentified during the field

282   sampling of the 23 sites. The 90%-quantile regression of Shannon diversity index (or

283   Hill number of order 1) against IBC_{metals} reveals a strong reduction in the upper level of

284   taxonomic diversity along the gradient of metal contamination ($p = 0.017$; Fig. 1B). No

285   significant relationship was found between the decreasing of Shannon diversity index

286   and the other physico-chemical parameters (Table S4). This result indicated that the

287   observed reduction of taxonomic diversity is not related to environmental parameters,

288   known to influence diversity. Similar results were obtained when species richness was

289   considered (Table S5).

290   According to the regional median value of Shannon diversity index

291   ($Shannon\ diveristy\ index = 9.41$; Table S6), two subgroups among uncontaminated

292   sites ($IBC_{metals} = 0, n = 10$, thereafter called "UnCont") can be distinguished. A first

293   subgroup UnCont1 ($n = 4$) with higher taxonomic diversity than the regional median

294   value ($Shannon\ diversity\ index > 10, mean = 11.9, sd = 1.9$), and a second subgroup

295   UnCont2 ($n = 6$) with lower taxonomic diversity ($Shannon\ diveristy\ index < 7$,

296     $mean = 5.4, sd = 1.0$). In addition, a group of sites ($n = 6$) were considered as

297   contaminated sites (thereafter called "Cont") because they had metal contamination

298   levels in the highest range of regional values (75%-quantile of regional $IBC_{metals} =$

299   0.256; Table 1; Table S7). Sites in Cont group exhibited also values of Shannon diversity

300   index lower than the regional median value. The observed reduction in the taxonomic

301   diversity between uncontaminated sites having the higher taxonomic diversity (i.e.,

302   UnCont1) and contaminated sites results in an average loss of 14 species.

303   To test the concurrent effect of metal contamination on the functional diversity of

304   macroinvertebrate assemblages, we focused on sites located at both ends of the

305   contamination gradient, i.e., UnCont1, UnCont2, and Cont groups of sites. Contrary to the

306   decreasing pattern in taxonomic diversity, no significant difference in functional

307   diversity values (i.e., FD_Q) is detected between the three groups of sites ($Df = 2$,

308    $F = 0.228$, $p = 0.799$; Fig. 1C). The PCoA representing the functional space occupied by

309   the whole set of species of the study area also underpinned this similarity in

310   macroinvertebrate functional diversity between contaminated and uncontaminated

311   streams: species occurring in UnCont1 ($n = 83$) occupy a large volume in this functional

312   space and overlap strongly with the species occurring in each of the two other groups of

313   sites ($n = 82$ for UnCont2 and $n = 69$ for Cont) (Fig. S4).

314   3.2.   Species replacement between uncontaminated and metal-contaminated streams

315   We then focused on the UntCon1 and Cont groups of sites to evaluate the processes

316   explaining the divergence of species assemblages along the drop of taxonomic diversity

317   associated with metal contamination. A significant deviation in the observed beta

318   diversity from the null expectation is detected when both incidence and abundance data

319   are used (incidence: $p = 0.0003$, abundance: $p = 0.0001$), revealing a non-random

320   spatial structuring of macroinvertebrate assemblages related to metal contamination at

321   the regional scale. Regarding the possible deterministic processes known to structure

322   spatial variability, incidence-based indices showed that the pattern of overall beta

323   diversity among UnCont1 and Cont group of sites ($\beta_{sor} = 0.54$) is mainly explained by

324   spatial turnover (76% contribution) relative to the nestedness of species assemblages

325   (24% contribution). In the case of abundance-based indices, the pattern of overall beta

326   diversity ($\beta_{BC} = 0.74$) is mostly caused by the balanced variation in abundances (84%

327   contribution) relative to the abundance gradients (16% contribution).

328   Furthermore, the pools of dominant species identified thanks to a taxon scarcity

329   metric appear to be dissimilar between Uncont1 and Cont (Fig. 2). Although these two

330   groups of sites share out 28 common dominant species (among 50 dominant species in

331   UnCont1 and 41 in Cont), different dominant species are specific either to

332   uncontaminated or contaminated sites ($n = 22$ species for UnCont1 and $n = 13$ species

333   for Cont). These UnCont1- and Cont-specific species are either dominant in one group

334   but not in the other, or occurred exclusively in one group of sites (Fig. 2). Besides, the

335   PCoA analysis describing the distribution of species throughout their trait-based

336   functional space (Fig. 3A) reveals that the 28 common dominant species are found in

337   seven of the ten functional groups identified by a cluster analysis of biological traits of

338   the whole species data set (Fig. S5; Table S3), tested by a MRPP analysis (see Materials

339   and Methods and Fig. S1) and exhibiting specific trait combinations (Fig. S2). Twenty

340   UnCont1-specific dominant species (among 22) co-occur with Cont-specific dominant

341   species in eight of the ten functional groups, and the two remaining UnCont1-specific

342   dominant species occur in one single functional group (FG3). Symmetrically, only one

343   (among 13) Cont-specific dominant species is found in a functional group with no

344   UnCont1-specific dominant species counterpart (FG7). Interestingly, UnCont1- and Cont-

345   specific dominant species contributed at similar levels to functional richness (estimated

346   by the contribution metric) and functional divergence (estimated by the originality

347   metric) in their respective communities (Fig. 3B). Furthermore, the functional

348   dissimilarity among UnCont1-specific dominant species is moderately lower than that

349   among Cont-specific dominant species whatever the normalization procedure

350   considered (e.g., UnCont1: $\beta = 1.15$; Cont: $\beta = 1.28$; see Table S8).

351 **4.** Discussion

352 Studying the ecological impact of micropollutant contamination into freshwater

353 ecosystems is a societal concern (Bennett et al., 2015). Humans indeed depend, socially

354 and economically, on the services that these ecosystems provide, including the

355 production of food and the maintenance of hydrological cycles (Aylward et al., 2005;

356 Grizzetti et al., 2016; Hanna et al., 2018). Recently, the need for a fruitful exchange of

357 concepts and methods between ecologists and ecotoxicologists to improve our

358 understanding of the effects of micropollutant contamination on biodiversity has been

359 highlighted (Beketov and Liess, 2012; Gessner and Tlili, 2016). Therefore, taking

360 advantage of the reliable assessment of exposure of communities to contamination

361 offered by an active biomonitoring methodology (Alric et al., 2019) used in national

362 monitoring programs implemented by French water agencies and by considering

363 advanced concepts from community ecology, we investigated at a regional spatial scale

364 how bioavailable metal contamination affects the integrity of stream macroinvertebrate

365 communities. Based on 23 study sites presenting a gradient of metal contamination,

366 comparable environmental conditions (altitude, size, hydroecoregion, absence of POPs

367 contamination), and harbouring a representative sample of the regional pool of species,

368 our study extends to the structural level of communities (i.e., the alpha diversity)

369 previous results, which already demonstrated the impact of bioavailable metal

370 contamination on the abundance of few specific macroinvertebrate species (e.g., Luoma

371 et al., 2010; Schmidt et al., 2011; Alric et al., 2019). The present observation of a decline

372 in the taxonomic diversity of macroinvertebrates in French watercourses confirms the

373 deleterious effect on regional freshwater biodiversity of metal contamination (Clements

374 et al., 2000), as demonstrated for pesticides (Beketov et al., 2013) and more generally

375 for other agents of global change (Peirera et al., 2012; Foley et al., 2005). We paid

376 attention to potential confounding factors in our analysis, and no significant effects of

377 other environmental parameters on the taxonomic diversity of macroinvertebrate

378 communities were detected among the 23 investigated streams. Nevertheless, our

379 assessment of non-metallic contamination is based on bioaccumulation data for 43 POP

380 compounds (including priority substances of the WFD; Alric et al., 2019). We have to

381 point that the vast majority of pesticides used in agriculture are not covered by this list

382 of monitored substances, even if some pesticides were considered (4,4'-DDD, 4,4'-DDE,

383 2,4'-DDT, 4,4'-DDT, dicofol, HCH γ (lindane), pendimethalin). So, we cannot fully rule out

384 the hypothesis of a confounding effect of pesticides could occur in our study region,

385 given that pesticides can have a non-negligible effect on structure of macroinvertebrate

386 communities (Beketov et al., 2013).

387   Contrary to the expectation of a congruent pattern between the two facets of

388 biodiversity (Bhin et al., 2010; Pool et al., 2014), the negative trend for taxonomic

389 diversity does not translate into similar trend for functional diversity (Fig. 1). This trend

390 follows previous works reporting that functional diversity may fluctuates quite

391 independently of taxonomic diversity in terrestrial communities (Cadotte et al., 2011;

392 Carmona et al., 2012) as in aquatic communities (e.g., macroinvertebrates; Reynaga et

393 al., 2013; Voß and Schäfer, 2017). Furthermore, similar to our results, two previous

394 works had also shown in their study sites that functional diversity was marginally

395 associated (Liess et al., 2021) or not (Voß and Schäfer, 2017) with environmental

396 pressures. It has been argued that the type of traits used for quantifying functional

397 diversity, the choice of functional diversity metrics, the level of functional redundancy,

398 the size of taxon pool and the strength of environmental filters can all shape the

399 relationships between these two facets of biodiversity (Cadotte et al., 2011). To estimate

400 functional diversity, we used the Rao's quadratic entropy considered as robust to

401 highlight differences in functional diversity in response to environmental pressures

402 (Brown and Milner, 2012; Gusmao et al., 2016). Similar results were obtained with the

403 functional dispersion index proposed by Laliberté and Legendre (2010) (Fig. S6 and Fig.

404 S7; see also Material S2). We also used the recommended type of functional traits (i.e.,

405 biological traits) reflecting the effect of organisms on ecosystem functions (Violle et al.,

406 2007), that are known to be sensitive to human-induced pressures (Statzner and Bêche,

407 2010; Lange et al., 2014), including micropollutant pollution (Archaimbault et al., 2010;

408 Pallotini et al., 2016). While, a small size of taxon pool has been highlighted as maying

409 restrict a strong functional response to environmental pressures (Voß and Schäfer,

410 2017), our study sites harboured a representative sample of the regional pool of species

411 (Fig. S3). An alternative reason for inconsistencies between the expected decreasing of

412 functional diversity and our field experimental result could be the insufficient amplitude

413 of the disturbance gradient (Gerisch et al., 2012), which may have been too short to

414 influence functional diversity. Nevertheless, the gradient of metal contamination studied

415 in the selected region ($0 \leq IBC_{metals} \leq 0.511$) fully covers the maximal range of

416 contamination levels observed at the French national scale (95%-quantile of national

417 $IBC_{metals} = 0.482$; Alric et al., 2019). This ensures the environmental relevance of the

418 findings of this regional pattern.

419   Different ecological mechanisms may explain the non-response of functional

420 diversity to the bioavailable metal contamination, in comparison with taxonomic

421 diversity reduction. Flynn et al. (2009) suggested that following a decrease in taxonomic

422 diversity in response to environmental degradation (e.g., land use intensification),

423 functional diversity may remain constant by a non-random mechanism corresponding to

424 the loss of functionally redundant species firstly. Behind this non-random mechanism,

425 there is the idea that resulting assemblages at impacted sites are subsets of the

426   assemblages encountered at unimpacted sites (Larsen and Ormerod, 2010). Rather than

427   supporting this nestedness pattern of species assemblages, our results show in

428   agreement with a recent meta-analysis (Soininen et al., 2018) that taxonomic-based beta

429   diversity was mainly driven by a non-random mechanism of spatial turnover. The

430   spatial turnover is here expressed not only as a shift in species dominance patterns

431   along the contamination gradient (in term of abundance, β_{BC}) but also as a replacement

432   of some species (β_{sor}). From a functional point of view, our analysis demonstrates that

433   the taxonomic turnover along the metal contamination gradient is coupled with a

434   compensation in the functional role (i.e., contribution to functional richness and

435   functional divergence) of dominant species (Fig. 3) considered as typical drivers of

436   ecosystem processes (Moore, 2006). Together these findings support the hypothesis

437   that the loss of functional diversity following the loss of sensitive species due to the

438   increase in level of an environmental disturbance (here bioavailable metal

439   contamination) is balanced with the non-random replacement of species by functionally

440   similar species (Bellwood et al., 2006). Our results confirm those of a previous work,

441   which assumed that the lack of effect of pesticides on functional diversity observed in

442   their study sites was probably due to compensatory processes, where the loss of

443   sensitive species can be compensated by tolerant species (Liess et al., 2021).

444   Our empirical work provides evidence of a dynamic response of regional

445   macroinvertebrate community to change in bioavailable metal contamination levels

446   with a decoupling between taxonomic and functional diversity. We documented a loss of

447   species but a maintenance of functional diversity relying on a process of non-random

448   species replacement by functional redundant species in contaminated streams. Our

449   results have important implications at the ecosystem level, as the maintenance of

450   functional diversity after species replacement can go hand in hand with the maintenance

451 of ecosystem functions (Little and Altermatt, 2018). Such decoupling underpinning the

452 stability of functional redundancy may strengthen the ability of macroinvertebrate

453 communities to withstand environmental change. Nevertheless, metal-tolerant species

454 would be expected to exhibit specific adaptations to metal toxic stress, which can go

455 with physiological costs or specific trade-offs between life history traits reducing their

456 ability to cope with other environmental disturbances (see Vigneron et al. (2015) for an

457 example of reduced feeding activity in a cadmium-tolerant population of the freshwater

458 shredder Gammarus). Considering such potential fitness costs of metal-tolerance, one

459 remaining question is therefore how far the observed functional resilience based on the

460 selection of peculiar metal-tolerant species among the regional pool of species is still

461 operant in multi-stressed aquatic environments.

462 5.   Conclusion

463 This study provides new insights into the consequences of bioavailable metal

464 contamination on the integrity of freshwater macroinvertebrate communities. While an

465 expected degradation in structural integrity of communities was observed in response

466 to increased bioavailable metal contamination, no significant changes were observed in

467 their functional integrity. Analysis of regional community structure supports the

468 hypothesis that niche-selection filtering rather than stochastic determines the functional

469 integrity of macroinvertebrate communities along the gradient of bioavailable metal

470 contamination, relying on a non-random process of species replacement by functional

471 redundant species. Such findings call for questioning the vulnerability of metal-tolerant

472 communities in multi-stressed environments. The underlying rationale is to test how

473 metal-tolerant species (i.e., Cont-specific dominant species) cope with, for example,

474 other types of pressures of human origin such as temperature stress, contamination by

475   POPs or pesticides, to assess whether functional diversity is maintained in multi-stress

476   contexts and to estimate consequences on ecosystem functions.

485   Author statement

486   Benjamin Alric: Conceptualization, Data Curation, Formal analysis, Investigation,

487   Methodology, Validation, Visualization, Supervision, Writing – Original Draft, Writing –

488   Review & Editing; Olivier Geffard: Conceptualization, Funding acquisition, Investigation,

489   Methodology, Resources, Writing – Review & Editing; Arnaud Chaumot:

490   Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing

491   – Review & Editing.

492   Competing Interests

493   The achievement of this manuscript has not been influenced by competing financial,

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500

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866 Figures

867 Fig. 1. Taxonomic and functional diversity along the metal contamination gradient. (A)

868 Map of the study watershed (Adour-Garonne, South West of France) with the geographic

869 location of sampling sites (dots) into the hydrographic network. In A, the insert

870 corresponds to the map representing the geographic delimitation of boundaries of six

871 French watersheds. (B) Shannon diversity index as a function of the metal

872 contamination (expressed from IBC_{metals}). Black solid line indicates the regression

873 fitted at 90th percentile distribution. Based on the AIC value, the linear model

874 ($AIC = 122.525$) was the best model to fit the tested relationships in relation to

875 curvilinear model ($AIC = 122.632$). The dots with a blue circle correspond to sites

876 belong to the first uncontaminated ($IBC_{metals} = 0$) group of site (i.e., UnCont1)

877 characterized by a high taxonomic diversity ($Shannon diversity index > 10$), whereas

878 dots circled in green belong to the second uncontaminated group of sites (i.e., UnCont2)

879 characterized by lower taxonomic diversity ($Shannon\ diversity\ index < 7$), and dots

880 circled in red belong to the contaminated ($IBC_{metals} > 0.26$) group of sites (i.e., Cont).

881 (C) Rao's quadratic entropy (FD_Q) estimated in uncontaminated (UnCont1 and

882 UnCont2) and contaminated (Cont) groups of sites. Colored dots represent Rao's

883 quadratic entropy for each sampling sites, whereas black triangles represent the average

884 Rao's quadratic entropy in each group of sites. Letters above each group of sites indicate

885 significant difference based on one-way analyses of variance (ANOVA) followed by

886 Tukey's HSD post-hoc test.

887 Fig. 2. Species sorted according to their average values of scarcity, A) in group of

888 uncontaminated sites (UnCont1) and B) in group of contaminated sites (Cont). To

889 simplify the interpretation, we used $1 - scarcity$, meaning that $1 - scarcity$ gets close

890 to one when the species dominates the assemblages and gets close to zero when the

891 species has low abundance. In each panel (A and B), species above the dashed horizontal

892 line had a $1 - scarcity$ higher than the 40th percentile and they are considered as

893 dominant species. Labels of these species are colored according to their occurrence in

894 the two groups of sites. Dominant species occurring specifically in UnCont1 are in blue

895 (panel A), whereas dominant species occurring specifically in Cont are in red (panel B),

896 and dominant species occurring in both groups are in black. In panel A, gold triangles

897 report the scarcity value of the dominant species in Cont occurring also in UnCont1.

898 Symbols * identified dominant species occurring exclusively in one group of sites, and

899 symbols ** identify species dominant in one group of sites but not dominant in the other

900 group.

901 Fig. 3. Species replacement and functional contribution between uncontaminated and

902 metal-contaminated streams. (A) Principal Coordinates Analysis (PCoA) representing

903 the functional space of macroinvertebrates based on their functional traits. The two axes

904 represent the two synthetic functional dimensions obtained from PCoA based on the

905 functional distance matrix (Euclidean distance). Each species is represented by a dot

906 colored: dominant species occurring exclusively in the uncontaminated group of sites

907 (UnCont1) in blue, dominant species occurring exclusively in the contaminated group of

908 sites (Cont) in red, dominant species occurring in both groups of sites (Common) in dark

909 grey, and rare species (Rare) in light grey. Convex hulls define groups of species with

910 different trait profiles. These functional groups (FG) were identified using a hierarchical

911 clustering analysis on the dissimilarity matrix (Euclidean distance) in the position of

912 species into the functional space shaped by the first two axes of PCoCA. See Table S2 for

913 the list of species and their memberships to the different functional groups. (B)

914   Functional contribution and functional originality for dominant species common (dark

915   grey) to uncontaminated (UnCont1) and contaminated (Cont) sites and for dominant

916   species specific (Uncont1: blue, Cont: red) to one of the two group of sites.
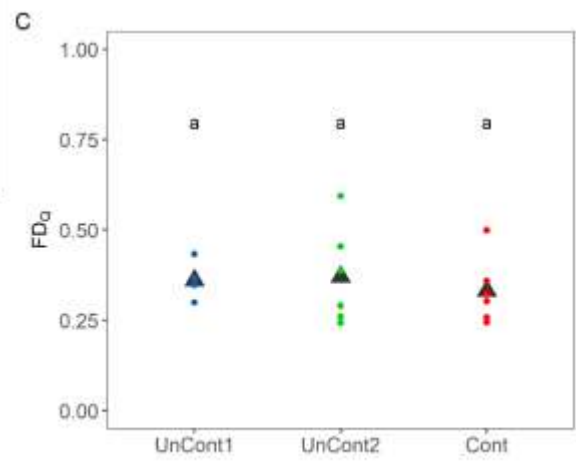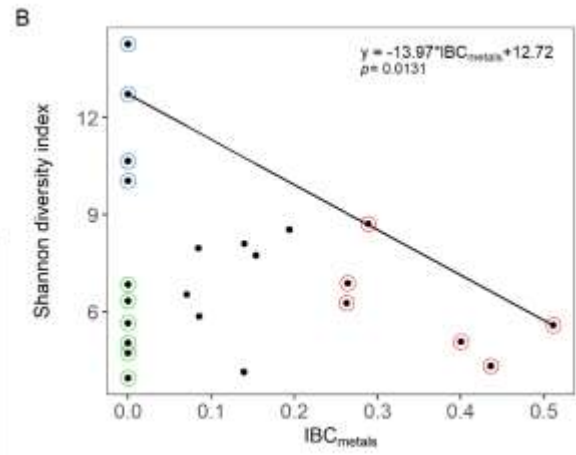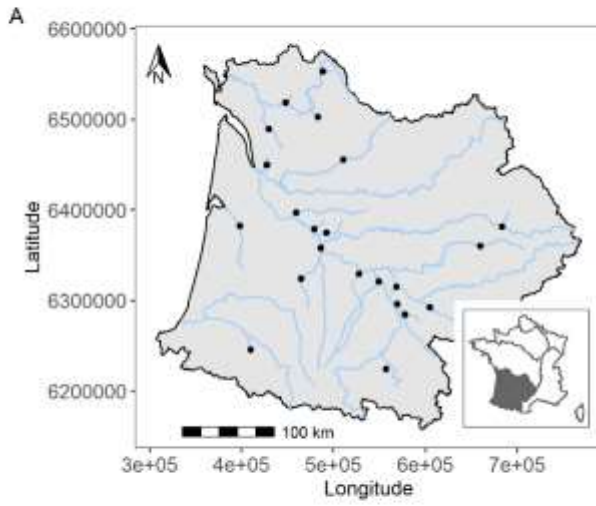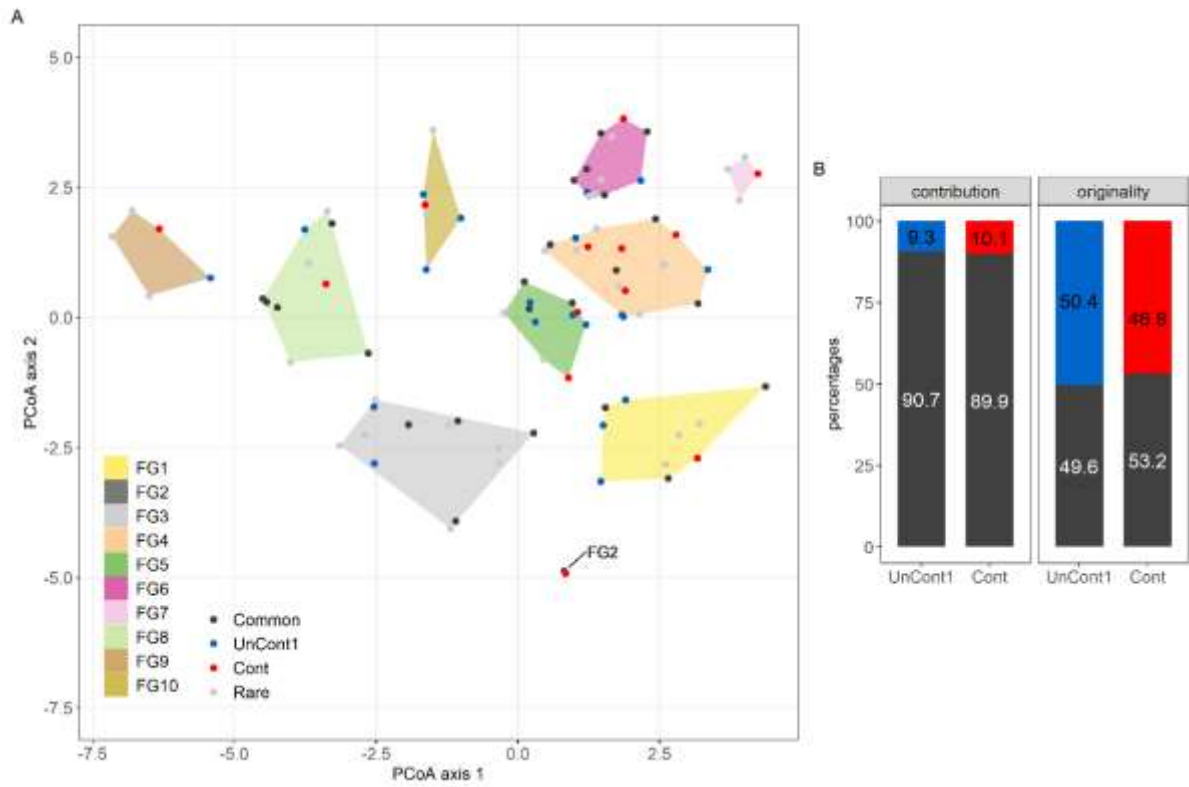
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918　Figure 1.
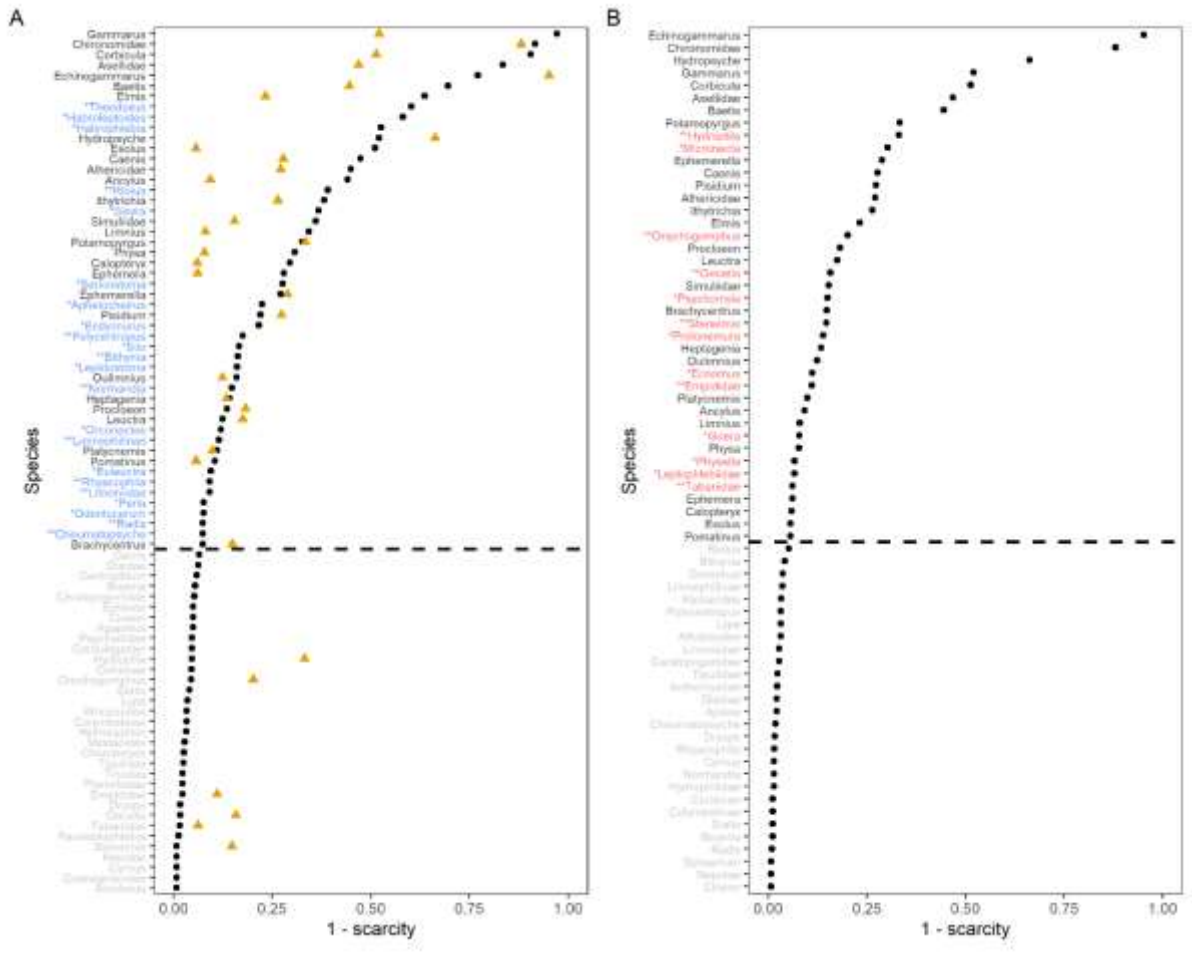
919
920   Figure 2.

921

922   Figure 3.

923

924   Table 1. Contextual characteristics of 23 sites (id) used in the study. The x and y

925   variables are the geographic coordinates estimated in Lambert93 projection system.

926   Elevation (altitude) is in meters. The strahler variable is the Strahler's stream order, a

927   positive integer used in geomorphology and hydrology to indicate the level of branching

928   in a river system. Hydroecoregion is a typology of streams considering a climatic,

929   hydrogeological, and landform hydroecological classification (Wasson et al. 2002).1:

930   Pyrénées (high mountain), 3: Massif Central Sud (mountain), 9: Tables Calcaires (plain),

931   11: Causses Aquitains (small relief), 13: Landes (plain), 14: Côteaux Aquitains (small

932   relief). IBC_{metals} is the bioavailable metal contamination level in each stream estimated

933   from the multi-substance indicator (Alric et al., 2019). In addition to the parameters

934   presented in the table, 20 other physico-chemical parameters were measured: BDO5, Ca,

935   Chl-a, Cl, Conductivity, Hydrometric Title (HT), K, Mg, Na, NH_4^+, NO_2^-, NO_3^-, O_2, pH,

936   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

937

938

Taxonomic diversity

Ecological process

Functional diversity

PCoA axis2
PCoA axis1

PCoA axis2
PCoA axis1

PCoA axis2
PCoA axis1

Micropollutant contamination

1