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1 Metal bioavailable contamination engages richness decline, species turnover but
2 unchanged functional diversity of stream macroinvertebrates at the scale of a French
3 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.

36 **1. Introduction**

37 With growing human population comes an increase in the diversity, production (e.g.,
38 industrial activities, mining) and release (e.g., urban or agricultural runoff) of chemicals
39 into freshwater ecosystems (Paul and Meyer, 2001; Carpenter et al., 2011). Hence
40 chemicals have become a major component of global environmental change, in the same
41 level of concern as other drivers such as atmospheric CO₂ concentration (Bernhardt et
42 al., 2017). Until recently, this global dimension of chemical contamination was not well
43 appreciated (e.g., Steffen et al., 2015; Persson et al., 2022), which may partly explain why
44 micropollutant pollution is one of the least-studied stressors in freshwater ecology
45 (Hevia et al., 2016). Among all micropollutants, trace metals are harmful environment
46 pollutants owing to their toxicity, non-biodegradability, and bioaccumulation (Hare,
47 1992; Stankovic et al., 2014).

48 Metal contamination can affect the health of ecosystems throughout adverse
49 effects on population dynamics and long-term adaptability of biological communities
50 (Ricciardi et al., 2009; Pereira et al., 2012). Field observations indicate that stream
51 macroinvertebrate assemblages are sensitive to metal contamination (e.g., Clements et
52 al., 2000), while they are a critical component for regulating the functioning of these
53 ecosystems (Vannote et al., 1980; Wallace and Webster, 1996). Typical documented
54 modifications include reduced macroinvertebrate abundance, reduced species richness,
55 and shifts in community composition from sensitive to tolerant taxa (e.g., Gower et al.,
56 1994; Clements and Kiffney, 1995; Maret et al., 2003; Cain et al., 2004; Liess et al., 2017;
57 Costas et al., 2018). Relationships have also been shown between metal contamination
58 levels in biota and ecological impacts (i.e., abundance) on freshwater communities (e.g.,
59 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 et 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 diversity 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 diversity 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 UnCont1 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.

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492 Competing Interests

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

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866 Figures

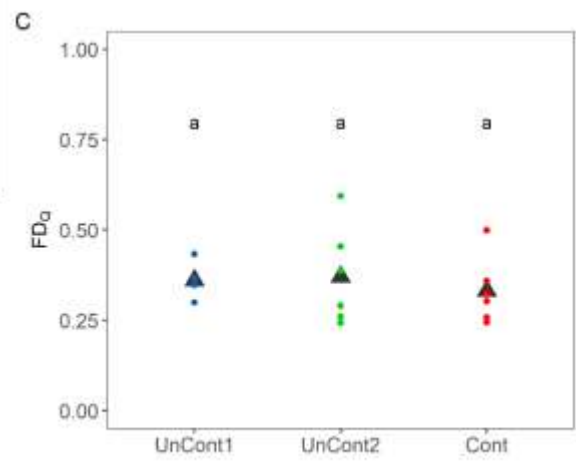
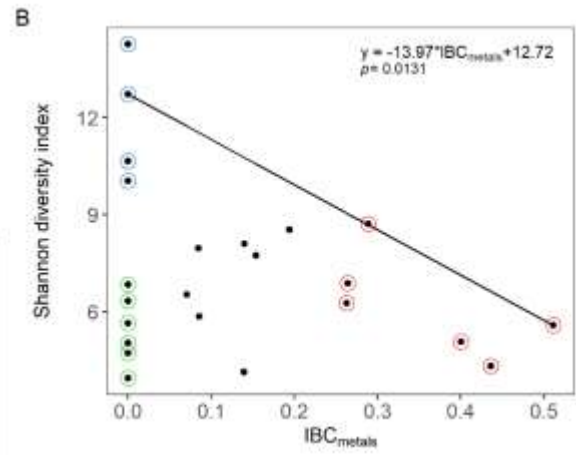
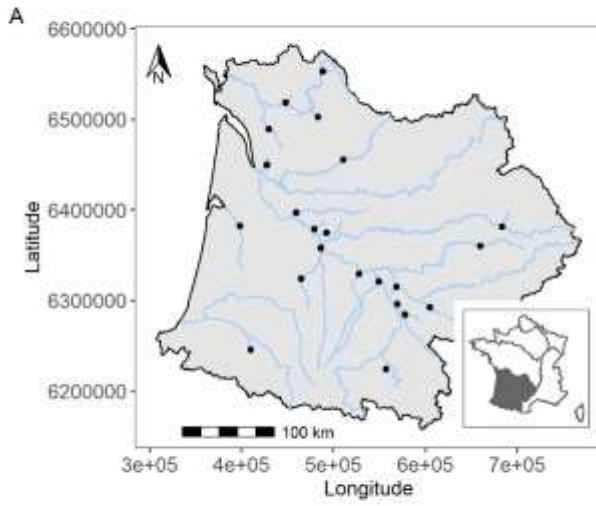
867 Fig. 1. Taxonomic and functional diversity along the metal contamination gradient. (A)
868 Map of the study watershed (Adour-Garonne, South West of France) with the geographic
869 location of sampling sites (dots) into the hydrographic network. In A, the insert
870 corresponds to the map representing 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.

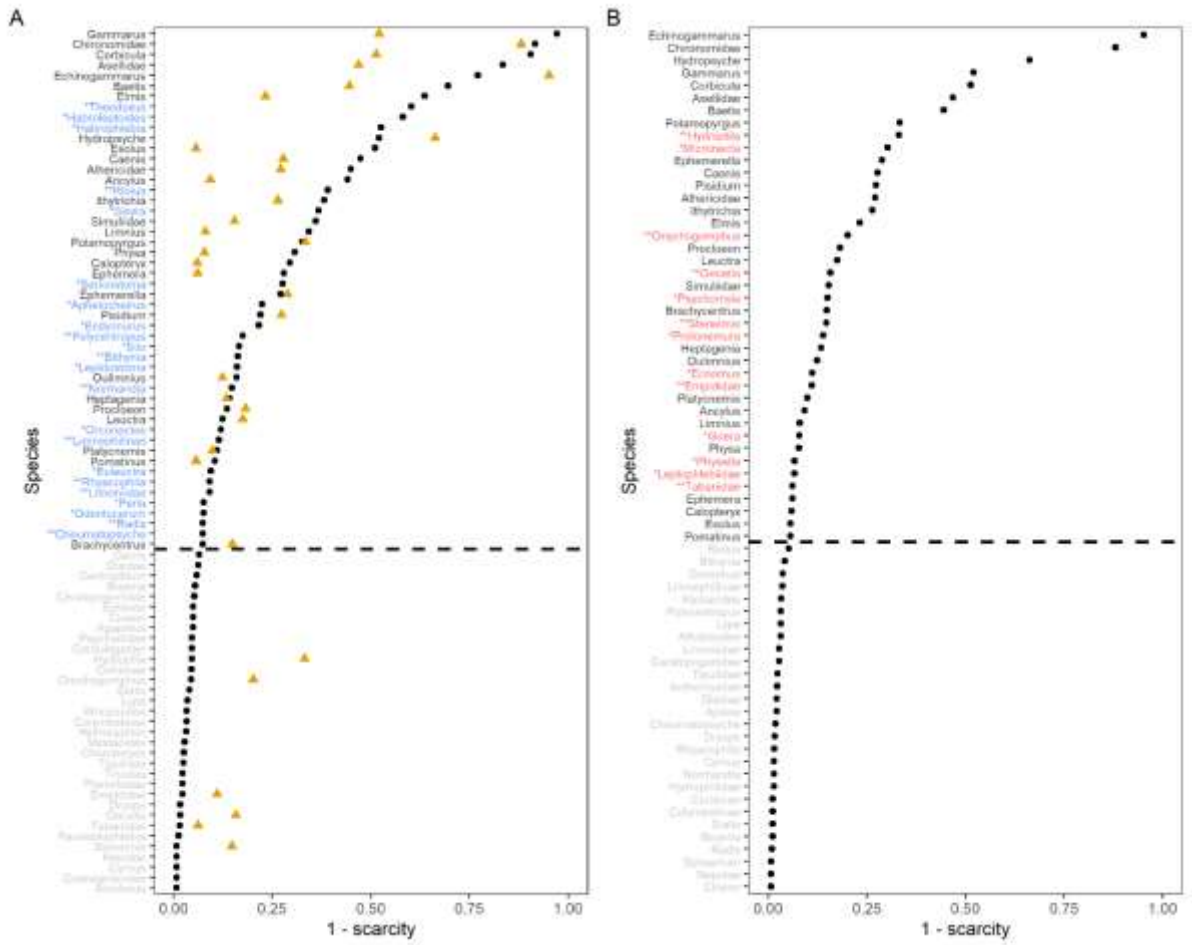
901 Fig. 3. Species replacement and functional contribution between uncontaminated and
902 metal-contaminated streams. (A) Principal Coordinates Analysis (PCoA) representing
903 the functional space of macroinvertebrates based on their functional traits. The two axes
904 represent the two synthetic functional 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.

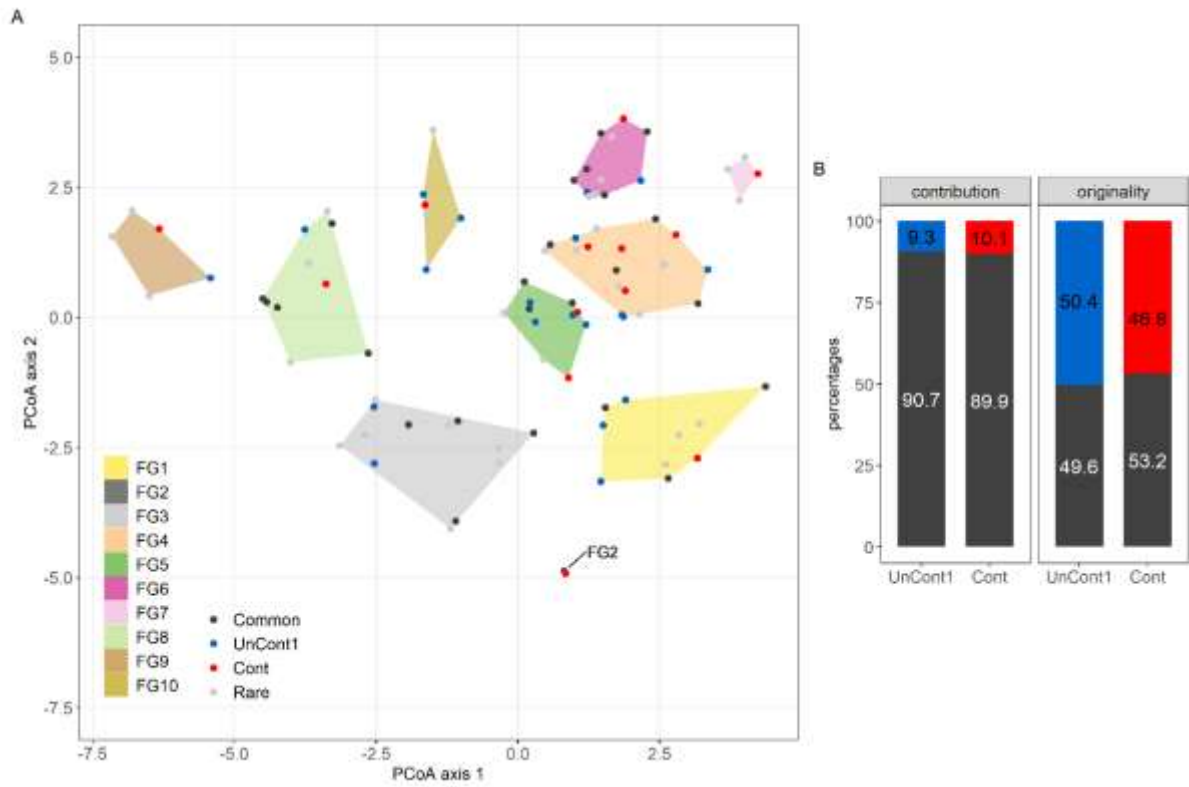


917

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	hydroecoregio	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

