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1 **Scale-dependency of macroinvertebrates communities responses to**
2 **contaminated sediments within run-of-river dams**

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16

17 **Summary**

18 Thanks to their nutrient recycling function and their importance in food-webs,
19 macroinvertebrates are crucial to the functioning of aquatic ecosystems. Because it
20 constitutes an essential compartment for biodiversity and functioning of aquatic
21 ecosystem, but also a potential source of contamination, sediment is an important
22 habitat to monitor and evaluate. In this context, we focused on macroinvertebrates
23 communities within run-of-river dams, that are privileged areas for sediment and
24 pollutants accumulation. Little is known about organization and response of littoral
25 macroinvertebrates communities within run-of-river dam, so we aimed to evaluate
26 (i) their functional and structural composition and (ii) the impact of pollutants
27 accumulation on them to select the most efficient scales and tools for the
28 biomonitoring of the contaminated sediments . Two run-of-river dams located in
29 French alpine area were selected and three spatial scales were examined :
30 transversal (banks and channel), transversal x longitudinal (banks/channel x
31 tail/middle/dam) and patch scale (erosion and sedimentation habitats). At the patch
32 scale, we evidenced that the heterogeneity of littoral habitats provides many
33 available niches, that allow the development of diversified macroinvertebrates
34 communities and thus highly variable responses to contamination. However, littoral
35 habitats, once pooled in the global "banks" spatial scale, spotlight the effects of
36 toxic disturbance .

37

38 **Keys words** : Contaminated sediment; Scale-dependancy; Invertebrates; Run-of-
39 river dams; Functional traits

40

41 **1. Introduction**

42 ***1.1 Research context***

43 If we consider that only 0.014% of Earth's Water occurs in the biosphere, water is a
44 limiting resource (Gleick, 1993). Increasing need of water resource for human
45 populations, agriculture and energy production has induced an excessive
46 development of dams or weirs and thus, many hydromorphological or chemical
47 disturbances of river systems (Zwick, 1992; Ward and Stanford, 1995; Bredenhand
48 and Samways, 2009). Owing to dam presence, the disruption of river continuity
49 (Stanford et al., 1996; Poff et al., 1997; Born et al., 1998) limits sediment transport
50 which accumulates within storages. This accumulated sediment becomes sink and
51 source of historic contamination. In fact, sediments have a strong pollutants
52 adsorption capacity (Cairns et al., 1984; Chapman et al., 1990; Estebe et al., 1997),
53 with contamination levels often greater than in the whole water column (Chapman,
54 1992). Moreover, when disturbed and resuspended, sediments can release adsorbed
55 contaminants into the water-column, that will be dispersed within the run-of-river
56 dam and in downstream ecosystems. In this context, historical contamination is a
57 real threat to the ecological integrity of many aquatic systems (Sheppard, 2005;
58 Norris et al., 2007; Johnston and Roberts, 2009).

59

60 ***1.2 Biomonitoring of contaminated sediment***

61 Contaminants stocked in the sediments exert a persistent pressure which implies
62 chronic and sublethal responses, with the alteration of macroinvertebrates
63 communities for instance (Field and Pitt, 1990; Beyer et al., 2000; Viganò et al.,
64 2002). However, the effects of those contaminants may extend beyond sediment
65 habitats, with their transfer to other compartments (Larsson, 1985; Asare et al.,

2000; Coulthard and Macklin, 2003). For all these reasons, biomonitoring tools were developed for contaminated sediment. Evaluation of sediments toxicity is essentially completed by biotests in laboratories but rarely account for responses at the community level (Lafont et al., 2007). However, because contamination can induce modifications in ecosystem's processes and thus, affect its integrity (Kiffney and Clements, 1994), it's necessary to focus on community's and ecosystem's levels. Thus, triadic studies that combine chemical, ecotoxicological and ecological approaches to evaluate sediment toxicity appear to be more appropriate tools, while still neglecting ecosystem functioning (Long and Chapman, 1985; Borgmann et al., 2001; Sorensen et al., 2007). Despite these challenges associated with sediment biomonitoring, storages, which are principally concerned by this topic, are rarely studied, partly because they are not considered within legal texts. This is particularly surprising, knowing that 60 000 dams are referenced in France. Since there is few knowledge of contaminated sediments' effects on macroinvertebrates communities (Ramsey et al., 2005), understanding the constraints on community structure and functioning in run-of-river dams is now an important challenge.

In this context, we aimed to assess structural and bio-ecological responses to contamination across several spatial scales, through different ecological indicators. We hypothesized that: (i) invertebrates communities should respond functionally and structurally to contaminated sediment but we forecast multiple responses according to the different spatial scales and (ii) when contamination is low, community response to pollutants should be overcome by habitat heterogeneity.

88

89

90

91 **2. Material and methods**

92 ***2.1 Sampling design***

93 Study area and contamination status

94 Two run-of-river dams of a French alpine river (the name of the river will not be
95 quoted because of the confidentiality agreements with the hydroelectric company
96 exploiting the dams) were selected in order to examine macroinvertebrates
97 composition associated with sediments' contamination. The catchment area is
98 dominantly industrial and urbanized inducing an accumulation of metallic
99 pollutants in the sediment of two successive hydroelectric dams. PCB and most of
100 PAH being below detection limits, the organic contamination is low.
101 Physicochemical parameters such as the concentration of dissolved oxygen, the
102 temperature and conductivity were also measured (Table 1). The data evidenced
103 different level of metallic contamination between the two systems.

104

105 Macroinvertebrates sampling

106 Macroinvertebrates were sampled in May 2008 with an original sampling protocol.
107 To distinguish influences of the different hydraulic and hydro-morphological
108 conditions, storages associated to run-of-river dams were subdivided in three areas
109 called "tail", "middle" and "dam". The tail is closer to river conditions, the dam is
110 typical of a lentic system and the middle exhibits intermediate characteristics.
111 Similarly, communities of the banks and of the channel were sampled separately.
112 Habitat's description was performed on each bank before sampling
113 macroinvertebrates communities with a surber net (mesh size 500 μm , sampled area
114 0.05m²). For the channel, the macroinvertebrates were sampled by dredging from a

115 boat perpendicularly to one transect for each of the three areas defined above. For
116 each transect, the left side, the middle and the left sides of the channel were
117 sampled. The samples were preserved in 4% formalin on the field and the
118 macroinvertebrates were sieved and sorted out at the laboratory, identified and
119 counted at the genus' level (Tachet et al. 2000) except for Diptera (family and tribe
120 level), Oligochaeta, and Nematoda.

121

122 ***2.2 Data analysis***

123 Three spatial scales were investigated: a) transversal scale– samples were pooled
124 according to their bank or channel origin – b) transversal x longitudinal scale –
125 samples were pooled according to the two factors considered together, for instance
126 banks x tail – and finally c) patch scale – samples were pooled according to the
127 habitat type (erosion, sedimentation or vegetation). For each scale, data were
128 explored through three types of indicators: structure indices, taxonomic
129 assemblages and bio-ecological traits structure.

130 Richness, abundance, proportion of main groups (EPT, Diptera, Molluscs, and
131 Crustacean), Shannon and Weaver diversity index, Simpson evenness and Rao
132 functional diversity index (Lavorel et al., 2008; de Bello et al., 2009), were
133 calculated. The functional diversity represents the overall difference among species
134 in a community according to their traits. We applied this index on the distribution
135 of categories within 22 traits (Table 2) describing biological and ecological traits of
136 macroinvertebrates (Usseglio-Polatera et al., 2000). At the transversal scale,
137 differences between run-of-river dams were investigated by a Principal Component
138 Analysis (PCA). At patches scales, the interaction between the two tested
139 parameters (habitat type and contamination status) were also assessed by an

140 ANOVA (normal distribution and homoscedasticity respected when the two factors
141 are considered), then Student's t-test and Mann-Whitney test were used to identify
142 the intra-factor differences (when factors are considered independently, parametric
143 conditions were not always respected). The vegetation habitat type was not
144 investigated here because the number of sampled substrates was too low in the
145 reference run-of-river dam.

146 Taxonomic composition and bio-ecological structure were also investigated. For
147 each spatial scale, the taxa's abundances were log-transformed and analyzed by
148 Correspondence Analysis (CA). For the functional structure, the biological and
149 ecological categories of traits were weighted by the log-transformed abundance of
150 each taxon. Therefore, for each trait category, the sum of weighted scores was
151 expressed as relative abundance distribution within a trait (Thioulouse et al., 1997).
152 We analyzed the frequencies of the categories using Fuzzy Correspondence
153 Analysis (FCA). We used R software (R development Core Team, 2008) and the
154 ADE4 library (Chessel et al., 2004) for all statistical analysis.

155

156 **3. Results**

157 ***3.1 Selection of relevant spatial scales***

158 To select the most relevant spatial scales, permutation tests were realized on the
159 multivariate analysis performed at the three spatial scales. They test the null model,
160 *i.e.* whether the structure of data is link to a particular factor (here the
161 contamination level) or is only the result of stochastic processes. The results are
162 synthetized in table 3. Statistical analysis reveals that the transversal scale
163 evidences significant faunistic and functional differences between the two stations,
164 while the patch scale discriminates the two stations only on the basis of indices.

165 Lastly, the transversal x longitudinal scale does not highlight inter-site differences.
166 For these reasons, only results obtained for transversal and patch scales will be
167 detailed below.

168

169 ***3.2 Response of indices on the two selected space scales***

170 Transversal scale

171 The first axis of the principal component analysis (Fig.1a) realized on index (table
172 4a) separates the reference banks from channels and disturbed banks. Observing the
173 results of correlations' circle (Fig.1b), this axis is strongly correlated with richness
174 and diversity index. Indeed, even if the banks offer more habitats than the channel,
175 the contaminated banks exhibit twice less species than the reference banks and on
176 average, as many species as in the channel. The same observation can be made with
177 the taxonomic and functional diversities. The EPT (Ephemeroptera, Plecoptera and
178 Trichoptera) and the Molluscs have suffered more from the disturbance. The second
179 axis of the PCA defines an abundance gradient positively correlated with reference
180 channel. Thus reference run-of-river dam is characterized by rich and diversified
181 littoral communities and high abundances in the channel. In contrast, the analysis
182 evidenced impoverished communities within disturbed banks. In spite of marked
183 reduction of abundance, disturbed channel seems less disturbed, the indices being
184 close to those of the reference channel. To some extent, at this level of
185 investigation, channels show structural similarities whereas banks index distinguish
186 both run-of-river dams.

187

188

189

190 Patches scale

191 The response of the richness, the abundance, the taxonomic and the functional
192 diversities, depending on two factors (contamination status and habitat type) was
193 tested by an ANOVA. The absence of a significant interaction between the factors
194 (table 5) permits us to test them independently. All variables respond significantly
195 to habitat type (Table 5). Conversely, at patch scale, only richness discriminates
196 toxic contamination status. Indeed, like table 4b shows, the sedimentation habitats
197 on the disturbed station are impoverished, that we can relate to the accumulation of
198 metals in the sediments.

199

200 ***3.3 Taxonomic against bio-ecological structure***

201 Transversal scale

202 The first axis of the correspondent analysis (Fig.2) shows differences of taxa
203 assemblages between reference and disturbed run-of-river dams. The second axis
204 reveals similarities between banks and channel in the reference system, while
205 differences are evidenced in the disturbed one. According to the fuzzy coding
206 analysis (Fig.3), the relative importance of the two factors (transversal localization
207 and contamination status) is inverted when compared to the taxonomic analysis.
208 Indeed the first axis shows that assemblages are functionally similar in banks and
209 channels in the reference system and different in the contaminated one while the
210 second axis separates the contamination level. Moreover, the percentage of
211 explained variance is higher with fuzzy coding analysis (77 % versus 44 %).

212

213

214 Patches scale

215 The main factor controlling functional analysis is the habitat type instead of the
216 contamination (Fig.4). The factorial plane F1F2 evidences that differences between
217 samples were governed by the habitat type, with discrimination along the F1 axis.
218 Conversely, within an habitat type, no differences are observed according to the
219 contamination status. In the same way, the analysis of faunistic assemblage doesn't
220 reveal significant responses. Finally, similarly to indices, at this scale, we show a
221 difference between habitats rather than between contamination status.

222

223 **4. Discussion**

224 ***4.1 Spatial scale***

225 Because observation scale governs our ability to detect and explain the
226 contamination impacts on macroinvertebrates communities, it is important to
227 determine which spatial scale, and which indicators (indices, taxonomic, bio-
228 ecological traits) are the most useful and efficient to detect the consequences of
229 contaminated sediments. This step is important to develop relevant tools for the
230 biomonitoring of this compartment.

231

232 Transversal scale

233 Our results showed the ability of banks' communities to identify contamination's
234 effects in contrast to those of channel. At this spatial scale, within-station substrate
235 heterogeneity effects are overcome by between-station differences associated with
236 contamination levels. Moreover, the habitat constraint is weaker in banks, with a
237 greater niche and macroinvertebrates communities diversity (Heino, 2000; Harrison
238 and Hildrew, 2001). It explains why banks are more prone than channel to differ

239 between sites with various contamination levels. Finally, being located at the
240 land/water interface and playing a key role in ecosystems' processes, the
241 knowledge of the littoral communities' structuration and of their functioning is
242 important. For example, in lakes, this area has been identified as an important
243 hydro-morphological component supporting ecological quality (Naiman and
244 Décamps, 1997; Rowan et al., 2006; Eloise et al., 2010). Free and colleagues
245 (2009), bring to light the close association of ecotone's integrity with the
246 requirements for adult life stages of mayflies. James (1998) insist on its importance
247 in overall production and in allochthonous carbon contribution to system.

248

249 Patches scale

250 In contrast with river system (Pardo and Armitage, 1997), patch scale wasn't
251 relevant to discriminate contamination effect. This result could be explained by the
252 extreme variability and complexity of patches communities which reduces the
253 sensibility of impacts detection (Donohue et al., 2009). Our study reveals that the
254 taxonomic and bioecological structures of macroinvertebrates communities were
255 mostly governed by habitat type, while the contamination only influences richness.
256 Since few studies focused on run-of-river dams, we can only compare our results to
257 those obtained in lakes or ponds. However, even in these systems, few studies have
258 focused on the use of littoral habitats for ecological assessment. Even if White and
259 colleagues (2003) recommend to base the sampling strategy on pebble or boulder
260 habitat, our results differed with none significant interaction between habitat type
261 and contamination. However, this could be explained by the analytical strategy, all
262 erosion habitats being pooled. Smaller scale should bring such information.

263 As Levin (1992) noted, “The key for understanding how information is transferred
264 across scales is to determine what information is preserved and what information is
265 lost from one scale to the other”. We observed that our ability to evidence a
266 response of communities to contaminated sediment depends on the level at which
267 we observe it. Our results suggest that focusing sampling effort on banks is more
268 relevant and practical for the biomonitoring of contaminated sediment.

269

270 ***4.2 Indicators scales***

271 For a long time, only taxonomic response to environmental variables and to the
272 contamination was evaluated with the consensus that the loss of species is
273 associated to a disturbance of the ecosystem. However, changes in the distribution
274 and in abundance of one species can result in disproportionate and unexpected
275 responses of other species resulting from an attempt to compensate functional
276 changes within the community (Naeem, 1998). This suggests that studying spatial
277 scale won't be enough, and that it is also necessary to wonder about relevant
278 indicators to identify and evaluate a perturbation. Indeed, when we use taxonomic
279 or functional indicators, we don't observe necessary the same response: the loss of
280 species doesn't systematically imply a response of the functional structure. In fact,
281 species may appear to perform the same function (i.e. be redundant) under a
282 restricted set of conditions, yet their functional roles may vary in naturally
283 heterogeneous environments (Walker, 1992; Wellnitz and Poff, 2001).. Traits are
284 one of the tools developed to investigate functional characteristics of a community.
285 By definition a trait is a surrogate of organism performance and/or individual
286 fitness (Violle et al., 2007). Numerous studies showed the ability of biological and

287 ecological traits to discriminate metallic (Archaimbault et al., 2010), hydraulic
288 (Snook and Milner, 2002; Griswold et al., 2008) or organic (Charvet et al., 1998)
289 perturbations. In the same way, even if we observed responses of taxonomic
290 structure to contamination, our results showed the relevance of bioecological
291 structure which is a better tool to evidence the impact of contamination in run-of-
292 river dam. However, this trait based approach assumes that these biological and
293 ecological parameters (autoecology of species) are relevant indicators of species'
294 functions inside the community. In fact, concept of trait finds all its interest when it
295 refers to functional trait (Chapin et al., 2000). Potentially, the traits defined by
296 Usseglio-Polatera et al. (2000) could be considered to be functional traits within
297 ecosystem's communities because their changes could directly affect ecosystem's
298 processes through changes in biotic controls (i.e predation) and indirectly through
299 changes in abiotic controls (i.e availability of limiting resources). For instance
300 species traits related to food resources and habits, habitat use and dispersal
301 capacities could be responses to contamination while driving changes in ecosystem
302 processes. In the same way, life history traits related to demographic parameters
303 (life cycle, fecundity, maximal size and number of generation) could be affected by
304 metallic contamination and thus indirectly alter ecosystem functioning by cascade
305 effects (Zavaleta et al., 2009). Traits used in this article are potential traits because
306 coming from literature, and are used as 'response trait', *i.e.* a measure of how the
307 traits assemblages react to environmental parameters at the community level. This
308 response may or may not be correlated with effects on functional processes, thus it
309 is necessary to discern and to be cautious on the notions of functional response trait
310 and functional effect trait (Lavorel and Garnier, 2002; Violle et al., 2007).
311 Forthcoming studies should also measure ecosystem's processes to compare

ecosystem functional response to community traits variation in order to identify whether functional traits of response and/or of effect to contaminated sediment could be defined.

4.3 Ranking impacts

Our results lead us to wonder about the hierarchy of the impacts, *i.e.* whether physical constraints could mask the effects of a moderate toxic contamination. Water flow governs the fundamental nature of streams and rivers (Poff et al., 1997; Hart and Finelli, 1999), so modifications of flow caused by dams alters the structure and function of river ecosystems (Malmqvist and Englund, 1996; Hart et al., 2002). However, there have been few studies that have examined the adverse effects of dams on the physical, chemical and biological characteristics of rivers (Poff and Hart, 2002). Presence of dams implies two perturbation types: 1) a physical disturbance by sediment accumulation and the modification of river flow; 2) a toxic disturbance, by potential pollutants accumulation within the sediments. The study of communities in the run-of-river dams can't dissociate these perturbations. The results obtained for the two studied systems revealed that habitats' characteristics of the channel decrease the differences related to contamination's level between the dams. However, no clear pattern is evidenced, highlighting the need to consider the dams and rivers continuum to discriminate these two sources of community modification.

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Figure 1

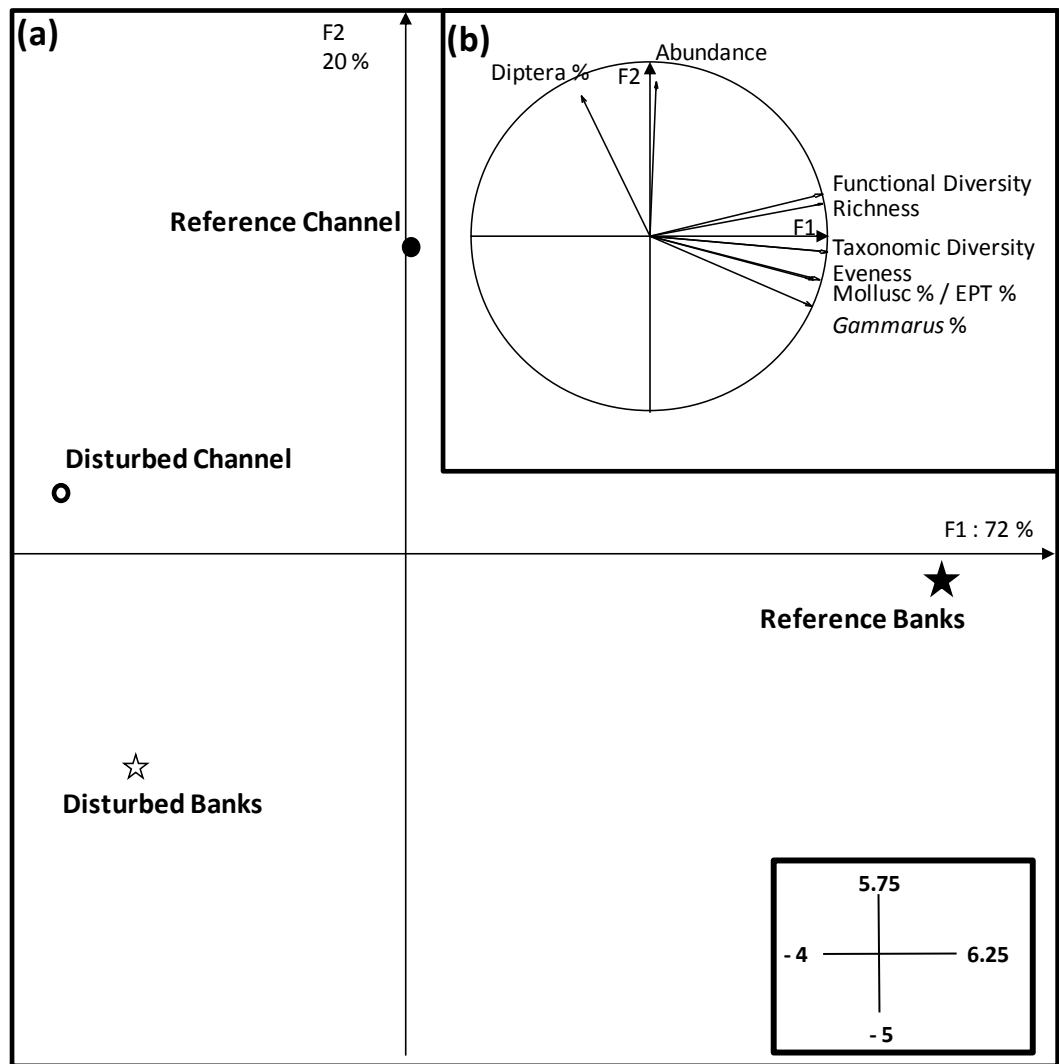


Figure 2

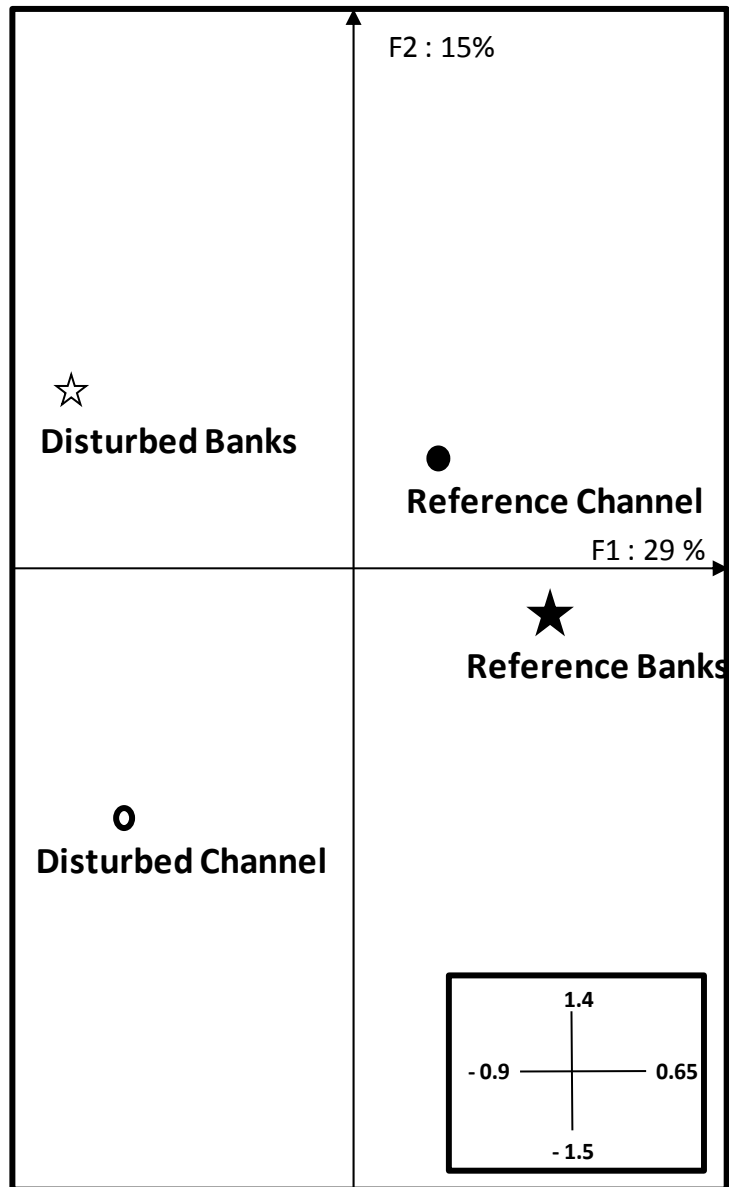


Figure 3

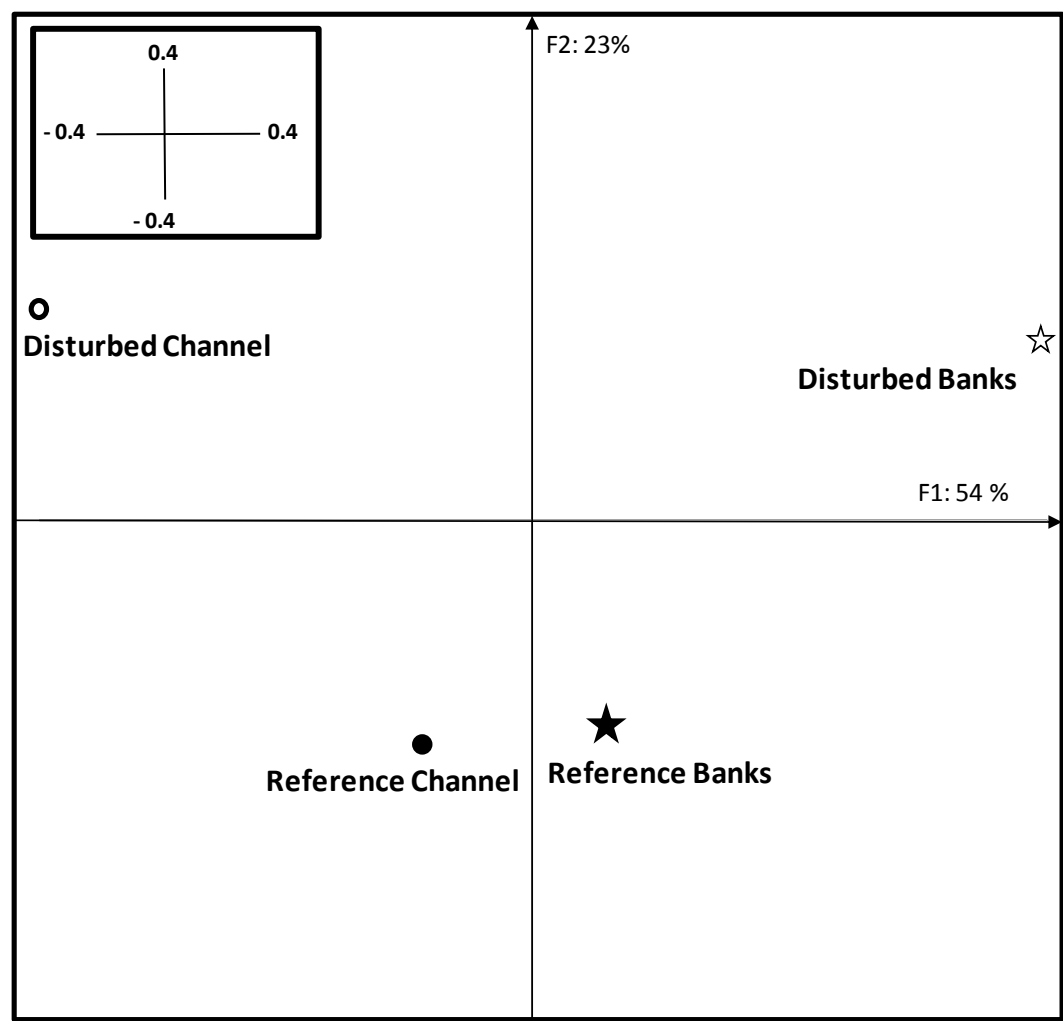


Figure 4

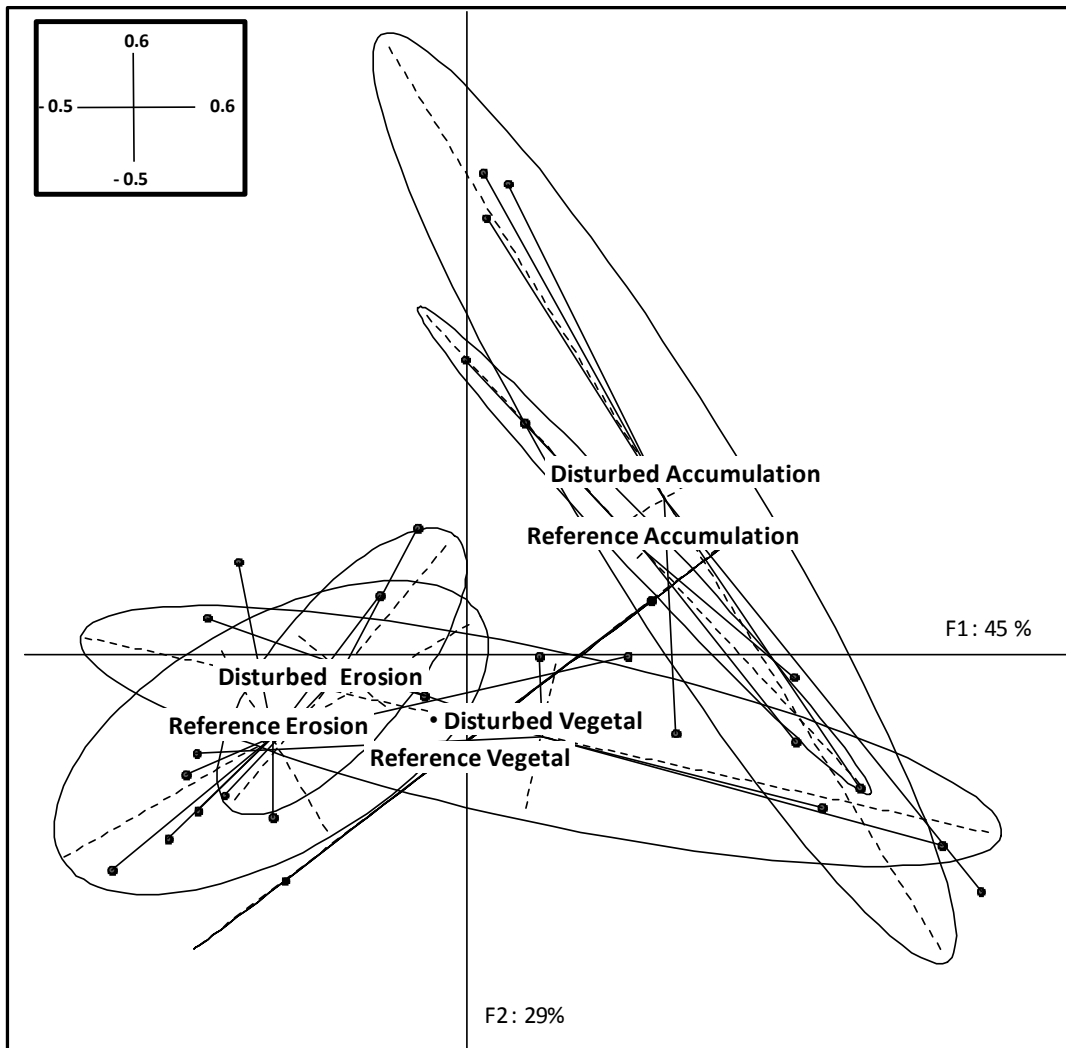


Table 1

Contaminants concentrations in sediments															
	Metals (mg.kgDW-1)										PAH (µg.kgDW-1)				
	Al	Fe	Cr	Co	Cu	Mn	Ni	Pb	Zn	As	BaP	BaA	BgP	Fluo	Ind
Reference site	9.2*10 ³	6.5*10 ³	38	4	14	169	7.7	22.5	90	1.9	18	24	27	101	24
Contaminated site	19.7*10 ³	15*10 ³	83.5	9	31	423	54.7	17.8	108	1	18	24	27	98	25

Water chemistry (mg.Γ ¹)												
	COD	DBO	Ca	Mg	K	NH4	Cl	NO3	NO2	SO4	PO4	pH
Reference site	1.9	1.5	70	5	2	0.32	15	16	0.28	15	0.218	8.2
Contaminated site	1.8	0.9	68	5	1.7	0.13	13	9.9	0.11	14	0.251	8.15

Table 2

Biological Traits	Categories	Ecological Traits	Categories
Maximal size (cm)	≤ 0.25	Transversal distribution	river channel
	> 0.25 to 0.5		banks, connected side-arms
	> 0.5 to 1		ponds, pools, disconnected side-arms
	> 1 to 2		marshes, peat bogs
	> 2 to 4		temporary waters
	> 4 to 8		lakes
Life span (year)	> 8	Longitudinal distribution	groundwaters
	≤ 1		crenon
	> 1		epirithron
Number of reproductive cycles per year	< 1		metarhithron
	1		hyporhithron
	> 1		epipotamon
Aquatic stages	egg		metapotamon
	larva		estuary
	nymph		outside river system
	adult		lowlands
Reproduction	Ovoviviparity	Altitude	piedmont level
	Isolated eggs, free	Biogeographic area	alpine level
	isolated eggs, cemented		Pyrenees
	clutches, cemented or fixed		Alps
	clutches, free		Vosges, Jura, Massif Central
	clutches, in vegetation		lowlands (oceanic)
Dispersal	clutches, terrestrial		lowlands (mediterranean)
	asexual reproduction		flags/boulders/cobbles/pebbles
	Aquatic passive	Substrate preferences	gravel
	Aquatic active		sand
Resistance forms	Aerial passive		silt
	Aerial active		macrophytes
	Eggs, statoblasts		microphytes
	Cocoons		twigs/roots
Respiration	Housings against desiccation		organic detritus/litter
	Diapause or dormancy		mud
	None	Current velocity	null
	Tegument		slow (< 25 cm/s)
Locomotion	Gill		medium (25-50 cm/s)
	Plastron		fast (> 50 cm/s)
	Spiracle	Trophic status	oligotrophic
	Hydrostatic vesicle		mesotrophic
Food	Flier		eutrophic
	Surface swimmer	Salinity (preferences)	freshwater
	Full water swimmer		brackish water
	Crawler		Psychrophilic (< 15°C)
Feeding habits	Burrower	Temperature	Thermophilic (> 15°C)
	Interstitial		Eurythermic
	Temporarily attached		Xenosaprobic
	Permanently attached	Saprobity	Oligosaprobic
Food	Microorganisms		β-Mesosaprobic
	Detritus (< 1mm)		α-Mesosaprobic
	Dead plant (>= 1mm)		Polysaprobic
Feeding habits	Living microphytes	Low pH sensitivity	≤ 4
	Living macrophytes		> 4 to 4.5
	Dead animal (>= 1mm)		> 4.5 to 5
	Living microinvertebrates		> 5 to 5.5
Feeding habits	Living macroinvertebrates		> 5.5 to 6
	Vertebrates		> 6
	Absorber		
	Deposit feeder		
	Shredder		
	Scraper		
Feeding habits	Filter-feeder		
	Piercer		
	Predator		
	Parasite		

Table 3

Scales				
		Indices	Faunistic assemblages	Bioecological traits
	Transversal	p-value = 0,001	p-value =0,001	p-value= 0,004
	Longitudinal x transversal	NS	NS	NS
	Patches	p-value = 0,001	NS	NS

Table 4

(a)	Banks		Channel	
	Reference	Contaminated	Reference	Contaminated
Richness	20 ± 8	10 ± 2	16 ± 6	10 ± 6
Abundance	746 ± 578	619 ± 604	4347 ± 4665	595 ± 733
EPT %	25 ± 24	5 ± 6	5 ± 8	12 ± 19
Diptera %	27 ± 18	22 ± 12	61 ± 26	52 ± 34
Mollusc %	3 ± 4	0.59 ± 0.84	1 ± 2	0.48 ± 0.78
<i>Gammarus</i> %	20 ± 21	17 ± 18	6 ± 8	6 ± 12
Functional diversity	0.72 ± 0.16	0.56 ± 0.29	0.56 ± 0.27	0.51 ± 0.28
Taxonomic diversity	2.57 ± 0.73	1.83 ± 0.96	1.83 ± 1	1.71 ± 1
Equitability	0.42 ± 0.12	0.30 ± 0.16	0.30 ± 0.17	0.28 ± 0.18

(b)	Erosion		Sedimentation	
	Reference	Contaminated	Reference	Contaminated
Richness	19 ± 6	15 ± 4	15 ± 4	7 ± 2
Abundance	1553 ± 3461	220 ± 81	3965 ± 3873	812 ± 733
Functional diversity	0.71 ± 0.26	0.81 ± 0.03	0.57 ± 0.06	0.33 ± 0.2
Faunistic diversity	2.61 ± 1	2.89 ± 0.28	1.70 ± 0.33	1 ± 0.65

Table 5

Variables	Interaction	Status factor	Habitat factor
Abundance	p = 0.44	p = 0.46	p = 0.03
Richness	p = 0.57	p = 0.01	p < 0.001
Taxonomic Diversity	p = 0.17	p = 0.17	p < 0.001
Functional Diversity	p = 0.06	p = 0.19	p < 0.001