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How can interspecific interactions in freshwater benthic macroinvertebrates modify trace element availability from sediment?

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**Credit author statement**

Andrade, Victoria Soledad: main investigation, and writing of the initial draft

Wiegand, Claudia: investigation, supervision, methodology, reviewing and editing of the manuscript

Pannard, Alexandrine: investigation, methodology, formal analysis, visualisation

Gagnetten, Ana María: funding acquisition, project administration, supervision,

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Piscart, Christophe: conceptualization, funding acquisition, project administration, supervision, investigation, methodology, providing of resources for the study, co-writing of the initial manuscript.

1 **How can interspecific interactions in freshwater benthic**  
2 **macroinvertebrates modify trace element availability from**  
3 **sediment?**

4

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19

20

## 21 **Abstract**

22 This study aimed to assess how bioturbation by freshwater benthic macroinvertebrates with  
23 different biological traits alone or in combination could modify trace elements (TE) fate  
24 between sediment and water, and if water TE concentration and animal TE content impair  
25 their body stores. Three macroinvertebrate species were exposed to TE contaminated  
26 sediment for 7 days: the omnivorous *Echinogammarus berilloni* (Amphipoda), the  
27 sediment feeding *Tubifex tubifex* (Oligochaeta) and the filter feeding *Pisidium* sp.  
28 (Bivalvia). Treatments were one without invertebrates (control), two with amphipods or  
29 mussels alone, and the combinations amphipod-mussel, and amphipod-mussel-worms.  
30 Water TE concentration increased significantly in 2 or 3 species mesocosms, concerning  
31 mainly Rare Earth Elements, Cr, U and Pb, known to be associated to the colloidal phase.  
32 By contrast, water soluble TE were not affected by animals. For both, amphipods and  
33 mussels, TE body content increased with the number of coexisting species. For amphipods,  
34 this increase concerned both, soluble and colloid-associated TE, possibly due to intense  
35 contact and feeding from sediment and predation on tubificids. TE bioaccumulation in  
36 mussel was less important and characterized by soluble TE, with water filtration as most  
37 plausible uptake route. Protein, triglyceride and Whole Body Energy Budget increased in  
38 amphipods with the number of coexisting species (probably by feeding on mussels' feces  
39 and tubificids) whereas triglycerides declined in mussels (presumably filtration was  
40 disturbed by amphipods). This study highlights interspecific interactions as key drivers  
41 explaining both: TE bioturbation, depending on their water solubility or colloidal  
42 association, and the exposure/contamination of species through another species activity.

43

44 **Keywords:** Bioturbation, Trace element, Macroinvertebrate, Rare earth element.

## 45 **1 Introduction**

46 Benthic macroinvertebrates bioturbate the sediment-water interface and are considered as  
47 ecosystem engineers because they modify this environment and influence the availability of  
48 resources and contaminants (Berckenbusch and Rowden, 2003). As many anthropogenic  
49 pollutants find their way into aquatic ecosystems and into the sediments as final sink  
50 (Eggelton and Thomas, 2004), bioturbation can modify their fate and distribution, either  
51 directly by mixing or resuspending subsurface and surface sediment, or indirectly by  
52 altering biogeochemical conditions (stability, redox conditions, organic content) (Blankson  
53 and Klerks, 2016).

54 Within the hydrographic network, trace elements (TE) are distributed as dissolved, colloid,  
55 or particulate form (Stumm and Morgan, 1996). Mineral constituents of sediments,  
56 particularly the clay fraction, exhibit large surface areas, thus huge sorption capacity  
57 regarding TE and organic compounds (Morley and Gadd, 1995). Moreover, redox-related  
58 biogeochemical processes facilitate the transition of the particle bound TE to the pore water  
59 and then to the pelagic zone (Xie et al., 2018). Remobilization of TE from sediments  
60 depends on many factors, including the physico-chemical characteristics of the TE itself,  
61 the content of organic matter and clay in the sediment, the hydrological flow, and non-the  
62 least the bioturbation by the benthic community (Blankson and Klerks, 2017; He et al.,  
63 2017, 2019; Xie et al., 2018).

64 Between TE, rare earth elements' (REE) characteristics make them sensitive tracers of  
65 water-rock interactions, groundwater mixing, past redox conditions and soil phases  
66 contributing to TE sources (e.g. Davranche et al., 2011; Laveuf et al., 2012; Pédrot et al.,  
67 2015). REE in aqueous solution have an organic speciation and complexation in the  
68 presence of natural organic molecules (e.g. Marsac et al., 2010; Pédrot et al., 2008),

69 exhibiting specific REE signatures (Davranche et al., 2005; Tang and Johannesson, 2010).  
70 Organic complexation of REE implies the development of a REE pattern in solution with a  
71 middle rare earth element (MREE) downward concavity, associated to an absence of Ce  
72 anomaly. Consequently, REE fingerprinting can allow to trace organic colloid  
73 solubilization (Pédrot et al., 2010) and can be used to distinguish a differential  
74 solubilization of organic colloids according to the activity of benthic communities.

75 Benthic macroinvertebrates bioturbate by feeding, locomotion, and using the sediment for  
76 their habitat (Pearson, 2001; Mermillod-Blondin and Rosenberg, 2006). Consequently,  
77 several functional groups have been proposed: biodiffusers, who randomly mix the  
78 sediment (e.g. amphipods); upward and downward conveyors, who move the sediment  
79 vertically through feeding activity; regenerators, burrowing organisms that relocate  
80 sediment (e.g. bivalves); and gallery diffusors, burrowing organisms that generate extensive  
81 tunnels (e.g. *Tubifex tubifex*) (François et al., 2002). Few bioturbation studies have focused  
82 on interspecific interactions affecting exposure to contaminants. For instance, *Lumbriculus*  
83 *variegatus* (Oligochaeta) bioturbation augmented zinc resuspension, but reduced its  
84 bioavailability for *Chironomus tepperi* (Chironomidae) (Colombo et al., 2016). Remaili et  
85 al. (2016) found an increase in metals by bioturbation with the number of coexisting  
86 species and a higher tissue concentration of chromium in bivalve in the presence of  
87 amphipods.

88 Benthic macroinvertebrates are good sentinels of pollution on freshwater ecosystems  
89 because of their relatively small action radius sensitivity to contaminants (Kiffney and  
90 Clements, 1994) and some with relatively long-life cycles (Rosenberg and Resh, 1993),  
91 moreover diverse ecological functions, thus taking part in a multitude of biological  
92 interactions (Gerino et al., 2003). Even though interspecific interactions are complex

93 (McAuliffe, 1984), it needs to be revealed how these diverse functional groups –  
94 individually or grouped – impact on contaminants fate, bioaccumulation and physiological  
95 effects.

96 To address these questions, three sediment-associated macroinvertebrates were selected: the  
97 omnivorous biodiffusor *Echinogammarus berilloni* (Amphipoda), the tunnel generating and  
98 sediment feeding *Tubifex tubifex* (Oligochaeta) and the burrowing and filter feeding  
99 *Pisidium* sp. (Bivalvia). *Echinogammarus berilloni* inhabits streams and rivers with a  
100 preference for running waters (Piscart et al., 2007, 2011). *Pisidium* sp. lives in a wide  
101 variety of habitats and seems to be of low sensibility towards high organic matter and  
102 decreased oxygen saturation (Herrington, 1962; Zeybek et al., 2012). *Tubifex tubifex*  
103 inhabits fine sediment in freshwater lakes or lotic river sections, it resuspends the upper  
104 sediment layer by feeding (Milbrink, 1987) and is relatively resistant to pollution  
105 (Karikhoff and Morris, 1985).

106 The aim of this study was to assess how bioturbation by freshwater benthic  
107 macroinvertebrates with different biological traits (i.e. omnivorous, filter feeder and deposit  
108 feeder) alone or in combination (single to three species) could modify TE distribution  
109 between sediments and water, using small mesocosms as exposure scenario. If bioturbation  
110 enhances bioavailability it might also cause physiological effects in the organisms exposed.  
111 Consequently, the specific goals were i) to analyze how macroinvertebrates bioturbation  
112 and their interspecific interactions affect the water TE concentration and the TE content in  
113 invertebrates; and ii) to analyze if water TE concentration and animal content impair body  
114 stores of macroinvertebrates.

115

116 **2 Material and methods**



### 117 2.1 Sediment collection

118 Sediment was collected using a grab sampler in the Sélune River (Normandy, France) at the  
119 confluence with the Yvrande River (48°34'24.0"N 1°11'04.8"W), a tributary known by its  
120 historical TE pollution caused by industries (DREAL-Normandie, 2012). The sediment,  
121 remaining wet in its water, was sieved ( $\emptyset$  2mm), homogenized and stored at 4°C two days  
122 before the experiment.

123

### 124 2.2 Organisms collection

125 *Echinogammarus berilloni* (Amphipoda) and *Pisidium* sp. (Bivalvia) were collected in the  
126 Petit Hermitage River (48°29'16.1"N 1°34'15.8"W) located in the Villecartier forest,  
127 considered as a reference site (Piscart et al., 2009). Specimens were maintained for one  
128 day in water of their origin at  $16.0 \pm 1^\circ\text{C}$  with aeration. *Tubifex tubifex* (Oligochaeta) were  
129 bought from a pet shop (Grebil Co., France) and maintained in the same conditions.  
130 Biofilm was collected in the Sélune River upstream of the confluence with the polluted  
131 Yvrande River using artificial substrates (glass plates) exposed for one month at 1m depth.  
132 Biofilm was then inoculated on glass gems (previously washed with HCl 1N and Milli-Q to  
133 remove TE) by incubating them during one month in a climate-controlled incubator with  
134  $30.0 \pm 1 \mu\text{mol m}^{-2}\text{s}^{-1}$  of light, 12:12 light:dark regime at  $20.0 \pm 1^\circ\text{C}$ .

135

### 136 2.3 Experimental design

137 Flat round glass bowls ( $\emptyset$ 14 cm) were filled with 100 mL of sediment and 200 mL of water  
138 from the reference site where invertebrates were collected. The approximate depth for  
139 sediment was 0.7 cm, and for water, 1.7 cm. The contact water/sediment surface was 154

140 cm<sup>2</sup>. The water/sediment proportion was chosen according to the number of organisms and  
141 their body sizes, and to ensure oxygen supply as no air bubbling was used to not add  
142 additional water current. The bowls were left to settle in a climate-controlled incubator  
143 (30±1 µmol m<sup>-2</sup>s<sup>-1</sup> light, 12:12 light:dark regime, 16±1 °C). After 24h, invertebrates and 5  
144 biofilm gems were added. Five combinations of organisms were performed with 5  
145 replicates each: one without invertebrates (control), two with 10 individuals of either  
146 amphipods or mussels alone, one combination of amphipod-mussel (10 individuals of  
147 each), and a combination amphipod (10 individuals) - mussel (10 individuals) - worms (20  
148 individuals).

149 The exposure lasted seven days to avoid any starvation (Foucreau et al., 2013) or oxygen  
150 depletion (Lawrence and Mason, 2001). From each bowl, one individual was frozen at -  
151 25°C for body stores and another specimen was dried at 65°C for 72h for TE content; four  
152 animals were frozen at -80°C for later enzyme analysis. Unfortunately none of the *T. tubifex*  
153 could be recovered. Samples of water and sediment were collected for nutrient  
154 concentration and TE content analysis.

155

#### 156 *2.4 Analysis of nutrients in the water*

157 Ammonium, nitrate and phosphate concentrations were measured at the beginning and at  
158 the end of the experiment from filtered water (GF/F) by colorimetric methods (Aminot and  
159 Chaussepied, 1983) using a Gallery Discrete Autoanalyzer (Thermo Scientific). The pH  
160 was measured with a combined Mettler InLab® electrode. Dissolved oxygen was measured  
161 with an Odeon portable apparatus (Ponsel) equipped with an optod probe.

162

#### 163 *2.5 Trace elements determination*

164 The elemental compositions of the sediment samples were determined at the CNRS Service  
165 d'Analyse des Roches et des Minéraux (SARM), Centre de Recherches Pétrographiques et  
166 Géochimiques (CRPG-CNRS, Nancy, France). After sample preparation (described in  
167 Carignan et al., 2001), the major element concentrations were determined by inductively-  
168 coupled plasma optical emission spectrometry (ICP-OES, Thermo ICap 6500) and the TE  
169 concentrations by ICP-mass spectrometry (Thermo Elemental - X7 ICP-MS). The  
170 bioavailable TE fraction in sediment was analyzed by leaching the water, acid soluble and  
171 exchangeable fraction of sediment, following the first step of modified BCR extraction  
172 (Pédrot et al., 2009). The first step was applied three times to 1 g of dried sediment sample,  
173 in ultracleaned 50-ml polypropylene centrifugal tubes. Extractant and procedure used: 0.11  
174 M  $\text{CH}_3\text{COOH}$ , 40 mL, room temperature, shake for 16 h, centrifuged 3000 g for 30 min.

175 All collected waters were filtered using 0.2- $\mu\text{m}$ -pore-size filter capsules (polyether sulfone  
176 membrane Sartorius Minisart). Major cation and TE concentrations were determined by  
177 ICP-MS (Agilent 7700x), using rhenium and rhodium as internal standard. Calibration  
178 curves and accuracy controls were performed in accordance with Yéghicheyan et al.  
179 (2013), using river water reference material for cation and trace element with a large  
180 compositional range (the international geostandard SLRS5, National Research Council of  
181 Canada). The water samples were solubilized in 0.37 M  $\text{HNO}_3$  with appropriate dilution(s)  
182 regarding the ICP-MS quantifications limits. At the beginning of the experiment, TE  
183 concentration were assessed in sediment (including the BCR), animals (supplementary data  
184 S1 and S2) and water; and at the end of the experiment, in water and animals.

185 For animal tissues, samples were prepared in a clean room in acid-washed digestion vessels  
186 (Savillex), respectively 50-mL tubes (24h in 1.5M  $\text{HNO}_3$  at 45°C, 24h in deionized water at  
187 45°C - repeated twice for the digestion vessels). Dry tissues were digested 5 times in sub-

188 boiled nitric acid (14.6 M HNO<sub>3</sub>), with or without hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), followed by  
189 8h evaporation (95°C) each time. Final solids were re-solubilized in 0.37 M HNO<sub>3</sub> with  
190 appropriate dilution(s) regarding the ICP-MS quantifications limits. Typical uncertainties  
191 including all error sources were below 5% for all TE, whereas for major cations, the  
192 uncertainty lied between 2% and 5%.

193

#### 194 *2.6 Body stores determination*

195 The concentrations in proteins, glycogen and triglycerides were measured *via*  
196 colorimetric assays, as described by Foray et al. (2012). Each individual was lyophilized  
197 and weighed (Balance XP2U Mettler Toledo, Columbus, OH, d=0.1 µg). A volume of 600  
198 µL of phosphate buffer was added to each sample, then homogenized for 1 min 30 sec at  
199 25Hz (bead-beating device, Retsch™ MM301, Retsch GbmH, Haan, Germany), and  
200 centrifuged (500 g, for 5 min, 4 °C). 2.5 µL of the supernatant were collected for  
201 quantifying protein content according to Bradford (1976) using bovine serum albumin as  
202 standard.

203 The rest of the supernatant (597.5 µL) was mixed with 900 µL of a methanol-chloroform  
204 solution (2/1, volume/volume). After separation of the phases by centrifugation at 4 °C at  
205 180g for 15 min, 300 µL of chloroform was transferred into new microtubes for triglyceride  
206 assays, and the pellets were kept for Glycogen content. Chloroform was evaporated using a  
207 speedvac (MiVac DUO, Genevac, France) before redissolving the remains in 400 µL of  
208 Triton-BSA buffer. The manufacturer's instructions were followed for the triglycerides  
209 colorimetric assay (Triglycerides, kit reference CC02200, LTA srl, Italy).

210 The glycogen content was assayed from the pellets by adding 1200 µL of fresh anthrone  
211 solution (1.42 g L<sup>-1</sup> anthrone in 70% sulfuric acid). Samples were heated at 90 °C for 15

212 min, and absorbance was measured at 625 nm after a 2-or 4-fold dilution. Glucose was used  
213 as standard.

214 The Whole Body Energy Budget (WBEB,  $\text{kJ.g}^{-1}$ ) was calculated as sum of combustion of  
215 ( $17 \text{ kJ.g}^{-1} \text{ glycogen} + 39.5 \text{ kJ.g}^{-1} \text{ triglycerides} + 24 \text{ kJ.g}^{-1} \text{ proteins}$ ) expressed per gram of  
216 dry weight of the sample (Smolders et al., 2003).

217

## 218 *2.7 Statistical analysis*

219 PCA was performed on the TE concentrations in water and the body content of  
220 invertebrates; dominant and physiologically relevant TE (Ca, K, Na, and Mg) were  
221 removed for the second PCA because they could be modified by physiological activities.

222 A PCA was also performed on nutrients concentrations, after scaling and reducing the data.

223 To highlight similar controlling processes (covariation, not causal-effect link) on nutrients  
224 and TE to treatments, the co-variations of nutrients and TE ordinations from the first two  
225 axes of PCAs were analyzed through a co-inertia (Doledec and Chessel, 1994; Dray et al.,  
226 2003). The expected link here is not a causal – effect one, but covariations, which would  
227 indicate similar controlling processes. The costructure between nutrients and TE is shown

228 on the plot of samples scores, on which the corresponding sample scores from both PCA

229 ordinations are linked by an arrow. The strength of the costructure is thus given for each

230 sample by the length of the arrow, with smallest arrows indicating maximum strength. To

231 test statistically the global strength of the costructure, a permutation test of Monte Carlo has

232 been performed by randomly permuting rows and columns of one PCA and calculating the

233 new co-structure (1000 permutations). All the analyses were performed using ade4 and

234 FactoMineR libraries implemented in R freeware (R Core Team, 2013).

235 The differences in nutrient or TE concentrations in water between treatments were assessed  
236 using one-way ANOVAs. The differences in TE body content and body stores were tested  
237 using two-ways ANOVAs with invertebrate species and species combinations as fixed  
238 factors. For all ANOVAs, Tukey's HSD tests were used to carry out post-hoc pairwise  
239 comparisons. All ANOVAs were performed using procedures from Statistica 7.1 (StatSoft,  
240 2004).

241

### 242 **3 Results**

243 In all mesocosms and treatments, the physico-chemical parameters remained suitable for  
244 the animals. The oxygen concentration remained above 7.0 mg.L<sup>-1</sup> and pH ranged from 7.0  
245 to 7.3 except for one mesocosm that increased until 7.7. For both, amphipods and mussels,  
246 survival rates were >90% in each mesocosm, except for one with only 80% of survival.

247

#### 248 *3.1 Nutrient and TE concentrations in Water*

249 Nitrate concentration increased significantly in the 2-species exposure ( $p < 0.008$ , Figure  
250 1). Ammonium and phosphate concentrations both increased significantly in the 3-species  
251 exposure ( $p < 0.0029$ ). Moreover, phosphate concentrations were significantly higher in all  
252 exposures containing amphipods ( $p < 0.003$ ); a similar tendency was also observed for  
253 ammonium.

254 The first component (PC1, Figure 2a) of the PCA of the TE concentrations in the water  
255 represent 54.6% of the total inertia and is explained by most of TE, whereas the second  
256 component (PC2) explains 16.3% variations of a group of 9 TE (B, Ba, Cd, Co, Li, Mn,  
257 Mg, Sr, and Ca). Eigenvalues are shown in Figure 2b. The water in mesocosms without  
258 invertebrates (controls) and the mesocosms with only one invertebrate species (mussels or

259 amphipods) had similar TE concentration, even though mesocosms with amphipod tended  
260 to have a higher content in TE associated with the second component (Figure 2c). The  
261 water of mesocosms with 2 or 3 invertebrate species had a significantly higher TE  
262 concentration than mesocosms with only one species (P-values < 0.05); for 76.7% of  
263 metals, these TE being associated with the first component. This group include REE, Fe,  
264 Cr, U, and Pb, which increased significantly with number of species (Figure 3a). Moreover,  
265 a MREE downward concavity was evidenced in all modalities, with an absence of  
266 evolution of Ce anomaly (data not shown). The increase of TE concentration is particularly  
267 strong for TE associated to organic colloids (e.g. Cr, Pb, REE and U), which are all on the  
268 right part of the PCA, whereas true dissolved TE (Li, B, Ba, Ca, K, Mg, Mn, Na and Sr)  
269 were not affected (Figures 2a and 3d,e). A co-inertia (Figure 4) has been performed from  
270 the previous PCA on TE concentrations and from a PCA on nutrients concentrations. Both  
271 ordinations showed a high percentage of variance on the first two axes, with 90 % for  
272 nutrients and 70 % for TE. The first axis of the co-inertia separates the three species  
273 treatments on the left part of the co-inertia from controls and mussels on the right part, with  
274 Amphipods and two species in the middle (Figure 4). Compared to the initial PCA, some  
275 TE are highlighted by the co-inertia, such as Rb for the first axis and Cu, Zn and As for the  
276 second axis (Figure 4). The dispersion of the samples on the plot moreover increased with  
277 the number of species.

278

### 279 *3.2 TE content in animal's body*

280 The mean TE content in non-exposed animals were relatively low. The ratios between the  
281 TE content in mesocosms with one species VS non-exposed animals ranged from 1 to 10  
282 for amphipods and from 0.4 to 2 for mussels.

283 The TE body contents for exposed animals are ranged between the two first components  
284 (PC1 and PC2) representing a total of 73.6% of the inertia (Figure 5a). The first component  
285 (PC1) represent 51% of the total inertia and is explained by most of TE, whereas the second  
286 component (PC2) explains 12.6% variations of a group of 13 TE (As, B, Ba, Cd, Co, Cu,  
287 Eu, Fe, Mn, Rb, Sb, Sr, and Zn). Eigenvalues are shown in Figure 5b. Apart from Ca,  
288 which is higher in mussels ( $F_{1,2,24} = 462.8$ ;  $P < 0.001$ ), the overall body content of TE was  
289 significantly higher in amphipods compared to mussels (Figure 5c). Concerning single TE,  
290 apart from Ni ( $P = 0.129$ ), and Cd ( $P = 0.454$ ), all others were significantly more  
291 concentrated by amphipods than by mussels ( $P$ -values  $< 0.003$ ). The body content in  
292 animals increased according to both, the species considered and the number of species in  
293 mesocosms. For amphipods, increase in TE concerned mainly TE associated with the  
294 second axis (e.g. Rb, Cu, Sr, and Mn), whereas for mussels this increase was mainly  
295 associated with the TE depicted on the first component (e.g. Al, Ce, Nd, and La) (Figure  
296 5c).

297 Amphipods' TE body contents were not correlated with TE concentration in water.  
298 However, for mussels, significant correlations, most of them being negative were found  
299 with 22 TE, including all REE, V Be, Cr, Fe, Ga, Pb, U, Y, and Zn.

300

### 301 *3.3 Body stores*

302 Protein content differed significantly between the species but not according to the  
303 treatments, despite a slight increase in amphipods with number of coexposed species  
304 (Figure 6a). Glycogen content did not differ significantly, neither between species nor  
305 between combinations of species (Figure 6b,  $P$ -values  $> 0.165$ ). Triglyceride contents did  
306 not differ significantly between amphipods and mussels in mesocosms with one or two



307 species (P-values > 0.276) but became significant in mesocosms with three species (Figure  
308 6c, c; P-values <0.018).

309 The WBEB was not significantly higher in amphipod than in mussels in mesocosms with  
310 one species (Figure 6d) but was significantly higher in the two and three species exposures  
311 (P = 0.004). TE concentrations in water did not significantly influence the WBEB, whereas  
312 significant negative correlations between WBEB and TE body content were observed. For  
313 amphipods, WBEB was negatively correlated with 22 TE (all REE, Fe, Ga, Pb, Sc, Si, U,  
314 V, and Y). For mussels, negative correlations were only found with Li (P = 0.027), Sb (P =  
315 0.036) and Sc (P = 0.043).

316

## 317 **4 Discussion**

### 318 *4.1 Nutrients and TE in Water*

319 Bioturbation of the upper sediment layer by amphipods and oligochaetes increased the  
320 concentration of ammonium and phosphate in the water, confirming studies by Mermillod-  
321 Blondin et al. (2008). Moreover, excretion by both organisms might have contributed,  
322 especially because animal biomass increased with the number of species in mesocosms  
323 (Fukuhara and Yasuda, 1989; Gardner et al., 1981; Nalepa et al., 1983). Amphipods are  
324 moreover suspected to have benefited from *T. tubifex* as nutrient source, thus their  
325 excretion and the decay of the oligochaetes contributed to the high values in these  
326 exposures. Contrarily, mussels alone did not change any of their energy storage, probably  
327 due to their lower activity in bioturbation.

328 Bioturbation similarly modified the TE content in the water, again in particular in  
329 exposures including amphipods. Li, B, Ba, Ca, K, Mg, Mn, Na and Sr, were not affected by  
330 the animals but crossed the benthic-pelagic border by diffusion. Indeed, these elements are

331 known to be not or slightly associated to the colloidal phase, resulting in a distribution  
332 within the truly dissolved phase ( $< 1$  nm) (Pédrot et al., 2008). Within the dissolved  
333 fraction in river waters ( $< 0.22$   $\mu\text{m}$ ), Fe-organic colloids are major carriers of TE with  
334 lower solubility (e.g. Cr, U, Pb and REE) (Pokrovsky and Schott, 2002; Sholkovitz et al.,  
335 1995). REE fingerprinting confirmed their organic complexation, and the increase of REE  
336 concentrations with the number of species showed an increase of organic colloid  
337 solubilization. Cr, U and Pb and REE were the most affected by the presence of organisms,  
338 indicating that bioturbation was the main factor releasing TE associated to colloids (less  
339 bioavailable) from the sediments. This study confirms that bioturbation contributes to TE  
340 redistribution from the sediment into the pelagic phase thus increasing its bioavailability. It  
341 moreover contributes to the understanding of the specificity of this process depending on  
342 the water solubility or colloidal association of the TE.

343 The contribution of each bioturbator type however, varied considerably. The least  
344 contributed the mussels, which even though living buried in the sediment seemed to have  
345 used the overlaying water for their respiration and filtering activities without causing  
346 disturbances of the sediment. The amphipods conversely, foraging by moving their  
347 sideways compressed body with their legs, apparently swirled sediment particles.  
348 Tubificids might have added to this bioturbation by their movements and moreover by  
349 feeding from deeper sediment layers and rejecting the feces into the overlaying space (He et  
350 al., 2019; Karlekhoff and Morris, 1985), but we could not prove it with our experimental  
351 set up. Similarly, comparing a range of benthic species, *Gammarus pulex* bioturbation  
352 activity was found to be higher than that of *T. tubifex*, which in turn liberated higher  
353 quantities of Cu from the sediment and considerably increased the toxicity (van der Meer et  
354 al., 2017).

355

356 *4.2 TE content in animal's body*

357 TE bioavailability to aquatic invertebrates is complex as both uptake routes (waterborne  
358 and dietary) exist in parallel (Brix et al., 2011). The dominant pathway may vary depending  
359 on species, permeable surfaces, feeding behaviour, metal, concentrations and available  
360 binding sites (De Jonge et al., 2010; Marsden and Rainbow 2004). Generally, amphipods  
361 seemed to accumulate more TE than mussels, confirming that they are physiologically good  
362 bioaccumulators (Geffard et al., 2010; Schaller et al., 2011). Amphipods foraging  
363 behaviour in intense contact to the sediment exposes their thin articular membranes and  
364 gills as uptake routes for TE. Secondly, feeding activities, especially predation on  
365 invertebrates such as oligochaetes (Piscart et al., 2011), tubificids in this scenario, also  
366 promotes their contamination with TE through biomagnification. Contrarily,  
367 bioaccumulation in mussels was lower and characterized by soluble TE (e.g. As and Zn),  
368 whose main uptake route was presumably by water filtration.

369 For both, amphipods and mussels, the whole TE content increased with the number of  
370 species in the mesocosms. TE affected by the experimental conditions were however not  
371 the same. In mesocosms with amphipods and mussels, the water soluble TE increased in  
372 amphipods which could be explained by the filtering activity of mussels, clearing the TE  
373 from the water column but concentrated them with their faeces near the sediment (Vaughn  
374 and Hakenkamp, 2001) where they are consumed by amphipods (Gergs and Rothaupt,  
375 2008). In those mesocosms, for mussels the soluble TE content decreased whereas colloidal  
376 TE content increased, according to its increase in the water caused by amphipods'  
377 bioturbation. Finally for both, amphipods and mussels, the increase in TE content were  
378 more important in the presence of tubificids. The tubificids' bioturbation activity might

379 have transferred TE from deeper layers of the sediment to the pelagic zone (He et al., 2019;  
380 Mermillod-Blondin et al., 2007; Reible et al., 1996), or modified physical properties of  
381 sediment, e.g. redox potential (Graf and Rosenberg, 1997), increasing their bioavailability.  
382 Moreover, for the amphipods increase could have been via biomagnification (Mermillod-  
383 Blondin et al., 2004). Our study confirms the increase of TE transport over the sediment  
384 water interface by bioturbation (Remaili et al., 2016) and moreover demonstrates how  
385 increasing interactions between invertebrates affect TE accumulation depending on species  
386 and TE partitioning between colloidal and dissolved.

#### 387 4.3 Body stores

388 In our exposure scenario with environmentally low TE concentrations, body stores of  
389 neither amphipods nor mussels were strongly impacted. On the contrary, body stores tended  
390 to increase with the number of species coexisting. This is consistent with previous studies  
391 showing a low effect of heavy metals on triglycerides and protein in the freshwater  
392 gastropod *Potamopyrgus antipodarum* exposed even at higher concentration of Cd (0.8  
393  $\mu\text{g.L}^{-1}$ ) and Zn (50  $\mu\text{g.L}^{-1}$ ) (Gust et al., 2011) than in our experiment (highest mean  
394 concentrations measured: Cd = 0.24 and Zn = 35.2  $\mu\text{g.L}^{-1}$ ). Moreover, amphipods and  
395 mussels are known to be good metal bioaccumulators able to sequester metals by cellular  
396 ligands such as metallothioneins, lysosomes, and mineralized organically based concretions  
397 without being considerably harmed (Geffard et al., 2010; Langston et al., 1998).

398 The correlation between protein and triglyceride contents with number of species exposed  
399 lead to a significant increase of WEBB in amphipods whereas it declined in mussels. This  
400 pattern highlights better experimental conditions for amphipods as they benefitted from the  
401 presence of tubificid preys (Mermillod-Blondin et al., 2004) and hence increased their  
402 energy. Amphipods might also have benefitted from mussels which concentrate the food

403 items (e.g. bacteria) in the surface of the sediment (Vaughn and Hakenkamp, 2001) or  
404 produce consumable feces (Gergs and Rothaupt, 2008). In turn, physical interactions  
405 between amphipods and mussels might have disturbed the filtration rate of mussels and  
406 lead to the observed decrease in their triglyceride content (and the low change in TE  
407 concentration). The pattern observed for glycogen in amphipods is more complex with a  
408 strong decrease in mesocosms with amphipods and mussels together. This might be due to  
409 the increase of certain TE in the water of these mesocosms, especially for the toxic ones  
410 such as As, Cd, Cu, and Zn (Borgmann et al., 2005). Glycogen is used by amphipods to  
411 cope with harsh environmental conditions and may strongly decrease in presence of toxic  
412 compounds (Dehedin et al., 2013; Maazouzi et al., 2011; Marmonier et al., 2013). The  
413 physiological state of the organisms in terms of their energetic stores was not that strongly  
414 impaired by the TE during the short exposure time (7 days) and the relatively low TE  
415 concentrations in the sediment. Nevertheless, some of the TE were negatively correlated  
416 with body stores of amphipods (Fe, Ga, almost all REE, Sc, Th, V, and Y) and mussels (B,  
417 Sb, Sc and Th). However, further studies are needed to understand the underlying  
418 mechanisms and the effects of TE over a longer term.

419

## 420 **5 Conclusion**

421 Our study confirmed the strong effect of bioturbation of sediments by aquatic invertebrates  
422 on the transfer of colloidal and organic matter associated TE to the pelagic compartment,  
423 whereas the soluble fraction of TE was not affected. We also highlighted that interspecific  
424 interactions appear to be a key driver explaining both the resuspension of TE, depending on  
425 their characteristic, and the exposure/contamination of species through another species  
426 activity. Previous studies combining several species with different trophic positions focused

427 on the biomagnification of TE along the food web, but our study revealed that a  
428 combination of several species would also control indirect contamination via increased  
429 bioavailability of TE by bioturbation activities. Moreover, we could not demonstrate high  
430 energy allocation in organisms exposed to TE but amphipods however, clearly benefitted  
431 from the additional energetic resources provided by tubificids. Further studies are required  
432 to measure the intensity of these interactions with more and or other functional feeding  
433 groups.

434

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442

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673 **Figure legends**

674 **Figure 1.** Mean ( $\pm$  SD) concentrations of nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>) and ammonium  
675 (NH<sub>4</sub>) in water according to the five experimental conditions. Significant differences are  
676 indicated by letters and numbers.

677

678 **Figure 2.** PCA on trace elements in water. (a) Correlation circle. (b) Eigenvalues. (c)  
679 Distribution of experimental conditions on the PC1–PC2 axes. Mean position of each  
680 experimental condition (white circles) are positioned at the weighted average of the five  
681 replicates (black dots).

682

683 **Figure 3.** Mean ( $\pm$  SE) of selected TE concentrations in water according to the five  
684 experimental conditions: (a) total content in Rare Earth Elements (REE), (b) iron  
685 concentration, (c) Sum of Cr, U and Pb, (d) total content in highly Soluble Trace Element  
686 (STE) including (Li, B, Ba, Ca, K, Mg, Mn, and Sr) , and (e) Sodium on its own.

687

688 **Figure 4.** Projection of two first axes of co-inertia analysis (COIA) after a double PCA: (a)  
689 nutrients concentrations, (b) TE in water (REE are shown in the box), and (c) samples  
690 scores by modalities and the arrows showing the change from one view to the other,  
691 indicating the strength of the links.

692

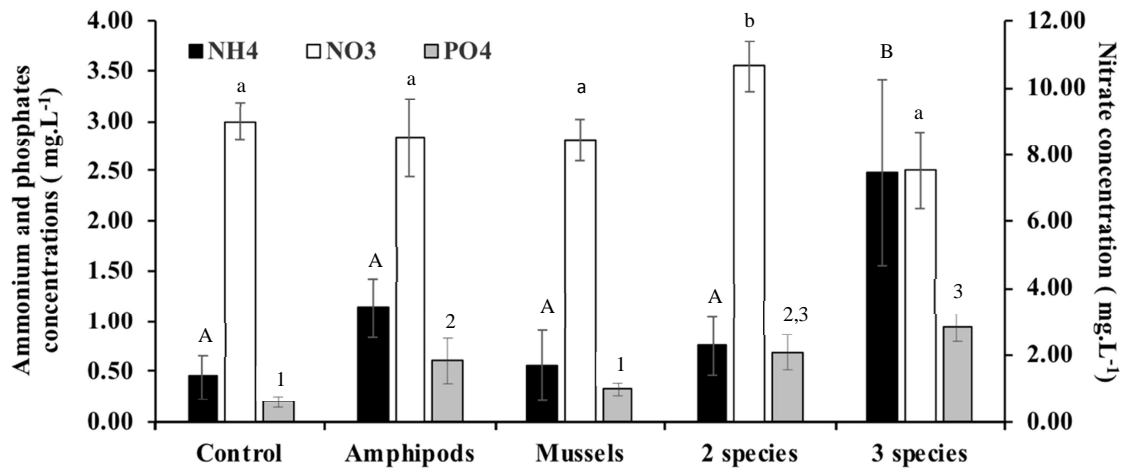
693 **Figure 5.** PCA on trace elements in animals: (a) Correlation circle, (b) Eigenvalues, (c)  
694 Distribution of experimental conditions on the PC1–PC2 axes. Mean position of each

695 experimental condition (white circles) are positioned at the weighted average of the five  
696 replicates (black dots).

697

698 **Figure 6.** Mean ( $\pm$  SE) content in (a) proteins, (b) triglycerides, (c) glycogen, (d) mean ( $\pm$   
699 SD) values for Whole Body Energy Budget (WBEB) according to experimental conditions  
700 for amphipods (dark bars) and mussels (white bars). Significant differences are indicated by  
701 letters.

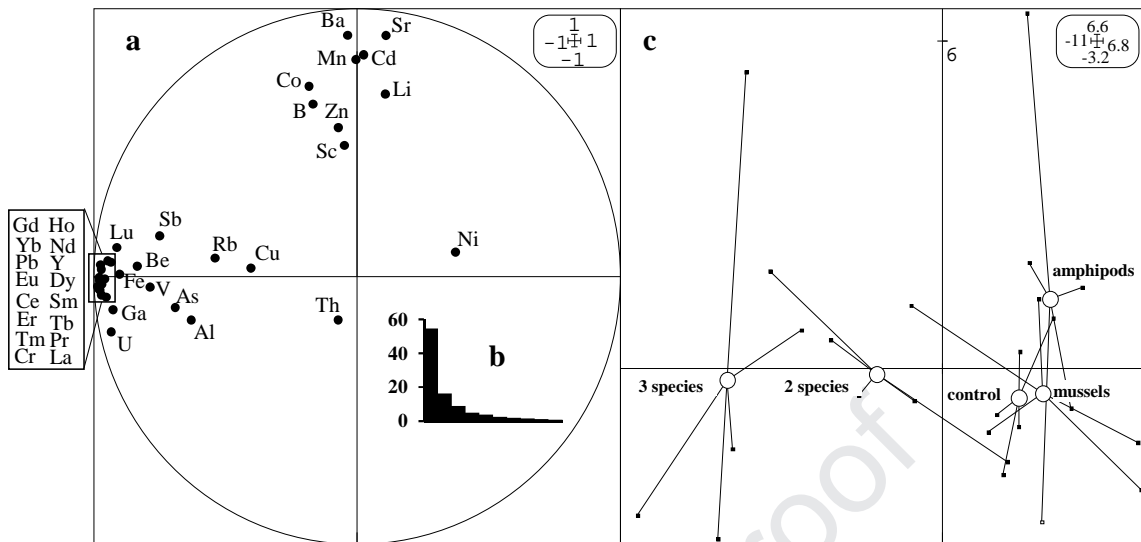
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704 **Figure 1.**

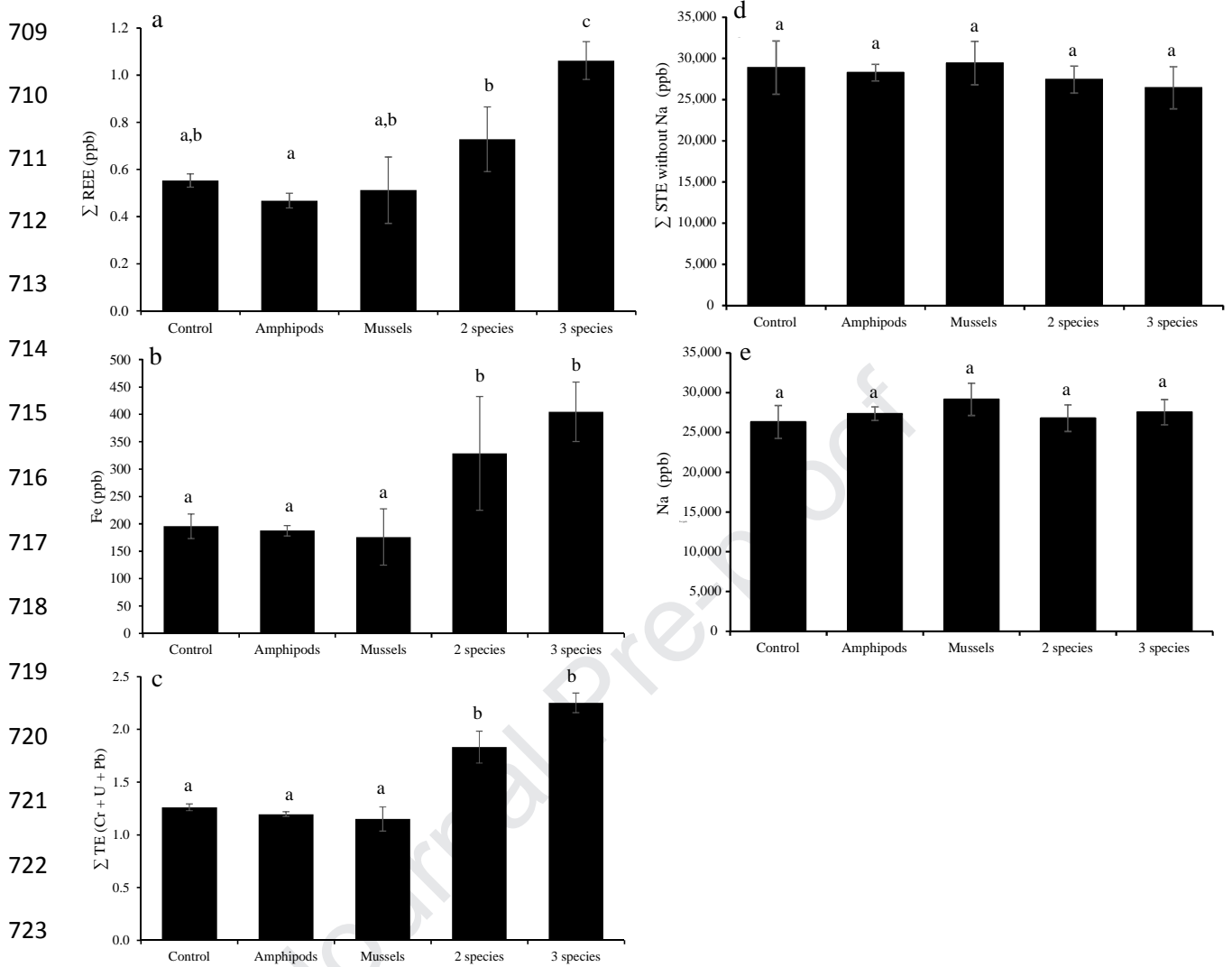
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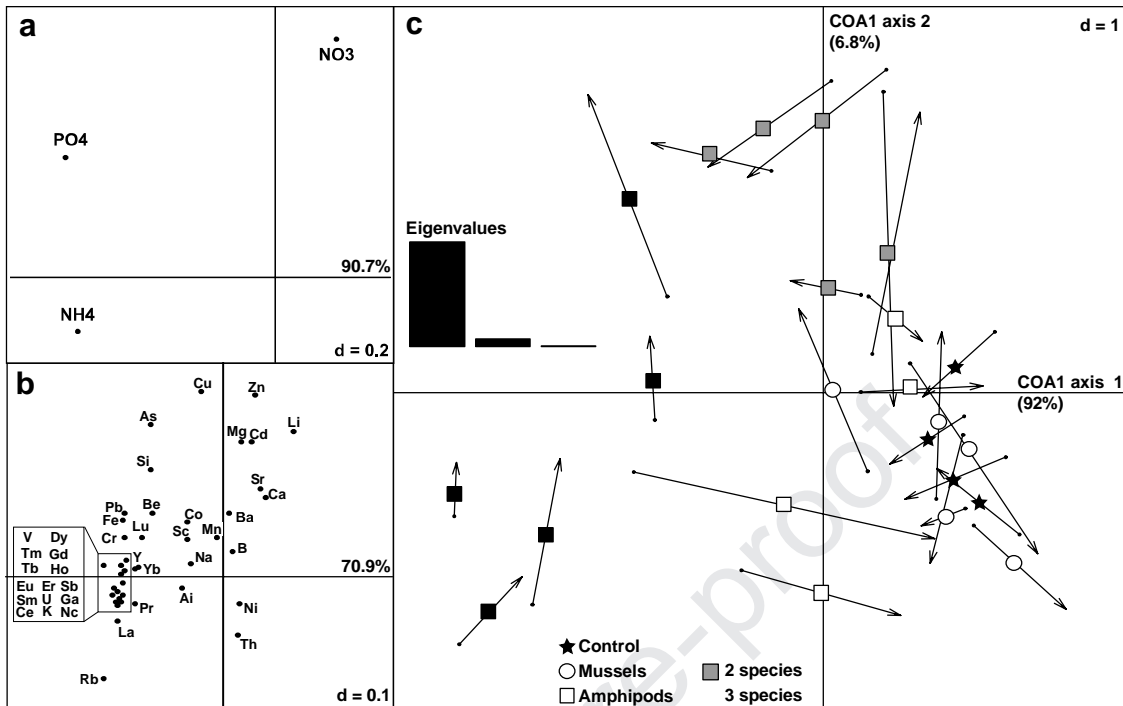


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707 **Figure 2.**

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**Figure 3.**

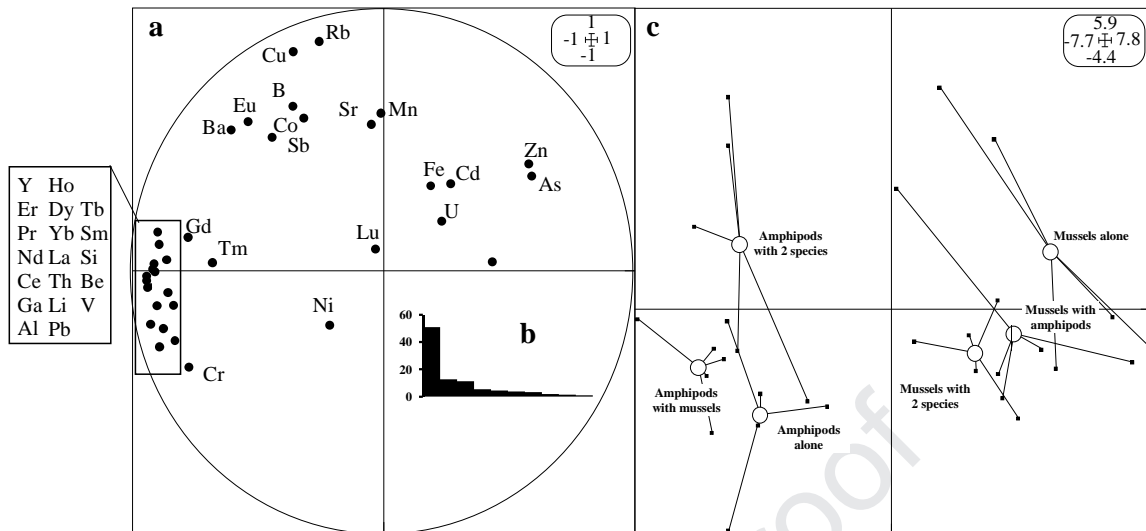


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728 **Figure 4.**

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731 **Figure 5.**

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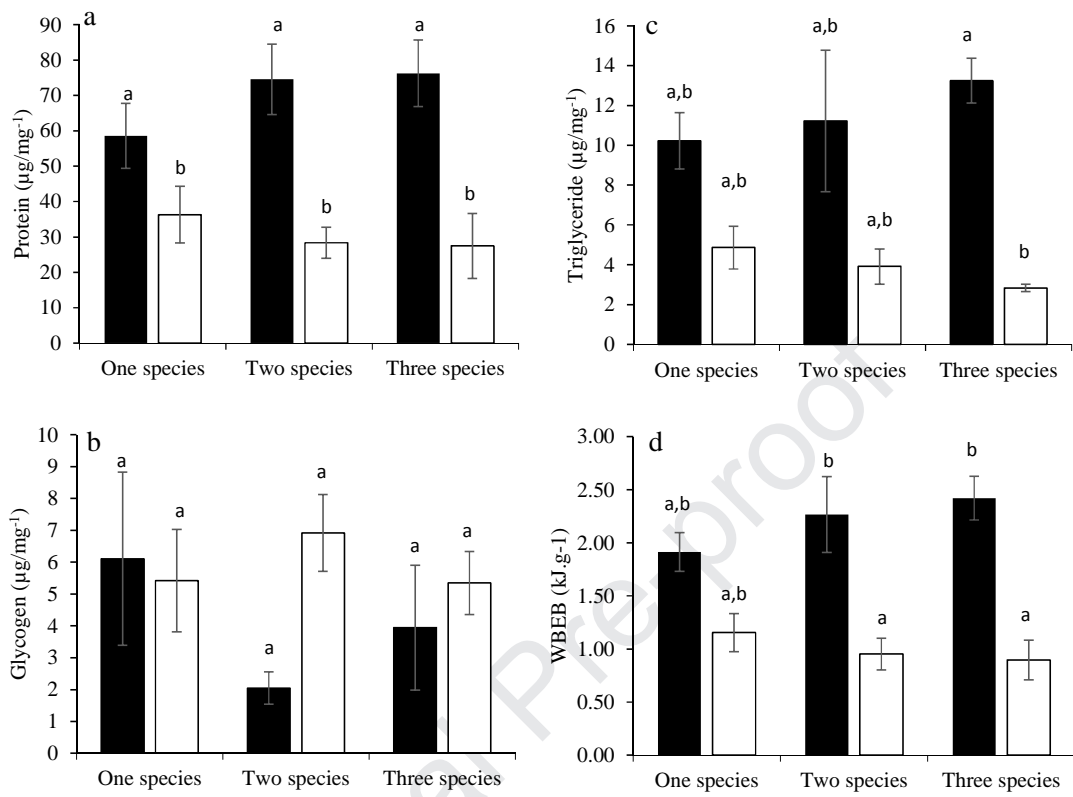


Figure 6.

**1 Highlights**

- 2 - Macroinvertebrates bioturbation affects trace elements fate among sediment and water
- 3 - Trace elements bioturbation depends on their solubility or colloidal association
- 4 - Trace elements bioturbation depends on macroinvertebrates biological traits
- 5 - Interspecific interactions are key drivers for trace elements bioaccumulation
- 6 - Bioturbators increase trace elements bioavailability for coexisting specie

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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