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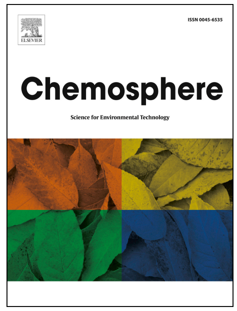

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How can interspecific interactions in freshwater benthic macroinvertebrates modify trace element availability from sediment?

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Abstract

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

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

1 Introduction

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

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

Between TE, rare earth elements' (REE) characteristics make them sensitive tracers of water–rock interactions, groundwater mixing, past redox conditions and soil phases contributing to TE sources (e.g. Davranche et al., 2011; Laveuf et al., 2012; Pédrot et al., 2015). REE in aqueous solution have an organic speciation and complexation in the presence of natural organic molecules (e.g. Marsac et al., 2010; Pédrot et al., 2008), exhibing specific REE signatures (Davranche et al., 2005; Tang and Johannesson, 2010). Organic complexation of REE implies the development of a REE pattern in solution with a middle rare earth element (MREE) downward concavity, associated to an absence of Ce anomaly. Consequently, REE fingerprinting can allow to trace organic colloid solubilization (Pédrot et al., 2010) and can be used to distinguish a differential solubilization of organic colloids according to the activity of benthic communities.

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

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

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

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

The aim of this study was to assess how bioturbation by freshwater benthic macroinvertebrates with different biological traits (i.e. omnivorous, filter feeder and deposit feeder) alone or in combination (single to three species) could modify TE distribution between sediments and water, using small mesocosms as exposure scenario. If bioturbation enhances bioavailability it might also cause physiological effects in the organisms exposed.

Consequently, the specific goals were i) to analyze how macroinvertebrates bioturbation and their interspecific interactions affect the water TE concentration and the TE content in invertebrates; and ii) to analyze if water TE concentration and animal content impair body stores of macroinvertebrates.

2 Material and methods

2.1 Sediment collection

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

2.2 *Organisms collection*

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

2.3 Experimental design

Flat round glass bowls (Ø14 cm) were filled with 100 mL of sediment and 200 mL of water from the reference site where invertebrates were collected. The approximate depth for sediment was 0.7 cm, and for water, 1.7 cm. The contact water/sediment surface was 154 cm^2. The water/sediment proportion was chosen according to the number of organisms and their body sizes, and to ensure oxygen supply as no air bubbling was used to not add additional water current. The bowls were left to settle in a climate-controlled incubator (30±1 µmol $m^{-2}s^{-1}$ light, 12:12 light:dark regime, 16±1 °C). After 24h, invertebrates and 5 biofilm gems were added. Five combinations of organisms were performed with 5 replicates each: one without invertebrates (control), two with 10 individuals of either amphipods or mussels alone, one combination of amphipod-mussel (10 individuals of each), and a combination amphipod (10 individuals) - mussel (10 individuals) - worms (20 individuals).

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

2.4 Analysis of nutrients in the water

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

2.5 Trace elements determination

The elemental compositions of the sediment samples were determined at the CNRS Service d'Analyse des Roches et des Minéraux (SARM), Centre de Recherches Pétrographiques et Géochimiques (CRPG-CNRS, Nancy, France). After sample preparation (described in Carignan et al., 2001), the major element concentrations were determined by inductively-coupled plasma optical emission spectrometry (ICP-OES, Thermo ICap 6500) and the TE concentrations by ICP-mass spectrometry (Thermo Elemental - X7 ICP-MS). The bioavailable TE fraction in sediment was analyzed by leaching the water, acid soluble and exchangeable fraction of sediment, following the first step of modified BCR extraction (Pédrot et al., 2009). The first step was applied three times to 1 g of dried sediment sample, in ultracleaned 50-ml polypropylene centrifugal tubes. Extractant and procedure used: 0.11 M CH_3COOH, 40 mL, room temperature, shake for 16 h, centrifuged 3000 g for 30 min.

All collected waters were filtered using 0.2-µm-pore-size filter capsules (polyether sulfone membrane Sartorius Minisart). Major cation and TE concentrations were determined by ICP-MS (Agilent 7700x), using rhenium and rhodium as internal standard. Calibration curves and accuracy controls were performed in accordance with Yéghicheyan et al. (2013), using river water reference material for cation and trace element with a large compositional range (the international geostandard SLRS5, National Research Council of Canada). The water samples were solubilized in 0.37 M HNO_3 with appropriate dilution(s) regarding the ICP-MS quantifications limits. At the beginning of the experiment, TE concentration were assessed in sediment (including the BCR), animals (supplementary data S1 and S2) and water; and at the end of the experiment, in water and animals.

For animal tissues, samples were prepared in a clean room in acid-washed digestion vessels (Savillex), respectively 50-mL tubes (24h in 1.5M HNO_3 at 45°C, 24h in deionized water at 45°C - repeated twice for the digestion vessels). Dry tissues were digested 5 times in subboiled nitric acid (14.6 M HNO_3), with or without hydrogen peroxide (H_2O_2), followed by 8h evaporation (95°C) each time. Final solids were re-solubilized in 0.37 M HNO_3 with appropriate dilution(s) regarding the ICP-MS quantifications limits. Typical uncertainties including all error sources were below 5% for all TE, whereas for major cations, the uncertainty lied between 2% and 5%.

2.6 Body stores determination

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

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

The glycogen content was assayed from the pellets by adding 1200 µL of fresh anthrone solution (1.42 g L-1 anthrone in 70% sulfuric acid). Samples were heated at 90 °C for 15 min, and absorbance was measured at 625 nm after a 2-or 4-fold dilution. Glucose was used as standard.

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

2.7 Statistical analysis

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

A PCA was also performed on nutrients concentrations, after scaling and reducing the data. To highlight similar controlling processes (covariation, not causal–effect link) on nutrients and TE to treatments, the co-variations of nutrients and TE ordinations from the first two axes of PCAs were analyzed through a co-inertia (Doledec and Chessel, 1994; Dray et al., 2003). The expected link here is not a causal – effect one, but covariations, which would indicate similar controlling processes. The costructure between nutrients and TE is shown on the plot of samples scores, on which the corresponding sample scores from both PCA ordinations are linked by an arrow. The strength of the costructure is thus given for each sample by the length of the arrow, with smallest arrows indicating maximum strength. To test statistically the global strength of the costructure, a permutation test of Monte Carlo has been performed by randomly permuting rows and columns of one PCA and calculating the new co-structure (1000 permutations). All the analyses were performed using ade4 and FactoMineR libraries implemented in R freeware (R Core Team, 2013).

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

3 Results

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

3.1 Nutrient and TE concentrations in Water

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

The first component (PC1, Figure 2a) of the PCA of the TE concentrations in the water represent 54.6% of the total inertia and is explained by most of TE, whereas the second component (PC2) explains 16.3% variations of a group of 9 TE (B, Ba, Cd, Co, Li, Mn, Mg, Sr, and Ca). Eigenvalues are shown in Figure 2b. The water in mesocosms without invertebrates (controls) and the mesocosms with only one invertebrate species (mussels or amphipods) had similar TE concentration, even though mesocosms with amphipod tended to have a higher content in TE associated with the second component (Figure 2c). The water of mesocosms with 2 or 3 invertebrate species had a significantly higher TE concentration than mesocosms with only one species (P-values < 0.05); for 76.7% of metals, these TE being associated with the first component. This group include REE, Fe, Cr, U, and Pb, which increased significantly with number of species (Figure 3a). Moreover, a MREE downward concavity was evidenced in all modalities, with an absence of evolution of Ce anomaly (data not shown). The increase of TE concentration is particularly strong for TE associated to organic colloids (e.g. Cr, Pb, REE and U), which are all on the right part of the PCA, whereas true dissolved TE (Li, B, Ba, Ca, K, Mg, Mn, Na and Sr) were not affected (Figures 2a and 3d,e). A co-inertia (Figure 4) has been performed from the previous PCA on TE concentrations and from a PCA on nutrients concentrations. Both ordinations showed a high percentage of variance on the first two axes, with 90 % for nutrients and 70 % for TE. The first axis of the co-inertia separates the three species treatments on the left part of the co-inertia from controls and mussels on the right part, with Amphipods and two species in the middle (Figure 4). Compared to the initial PCA, some TE are highlighted by the co-inertia, such as Rb for the first axis and Cu, Zn and As for the second axis (Figure 4). The dispersion of the samples on the plot moreover increased with the number of species.

3.2 TE content in animal's body

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

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

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

3.3 Body stores

Protein content differed significantly between the species but not according to the treatments, despite a slight increase in amphipods with number of coexposed species (Figure 6a). Glycogen content did not differ significantly, neither between species nor between combinations of species (Figure 6b, P-values > 0.165). Triglyceride contents did not differ significantly between amphipods and mussels in mesocosms with one or two species (P-values > 0.276) but became significant in mesocosms with three species (Figure 6c, c; P-values <0.018).

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

4 Discussion

4.1 Nutrients and TE in Water

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

Bioturbation similarly modified the TE content in the water, again in particular in exposures including amphipods. Li, B, Ba, Ca, K, Mg, Mn, Na and Sr, were not affected by the animals but crossed the benthic-pelagic border by diffusion. Indeed, these elements are known to be not or slightly associated to the colloidal phase, resulting in a distribution within the truly dissolved phase (< 1 nm) (Pédrot et al., 2008). Within the dissolved fraction in river waters (< 0.22 µm), Fe-organic colloids are major carriers of TE with lower solubility (e.g. Cr, U, Pb and REE) (Pokrovsky and Schott, 2002; Sholkovitz et al., 1995). REE fingerprinting confirmed their organic complexation, and the increase of REE concentrations with the number of species showed an increase of organic colloid solubilization. Cr, U and Pb and REE were the most affected by the presence of organisms, indicating that bioturbation was the main factor releasing TE associated to colloids (less bioavailable) from the sediments. This study confirms that bioturbation contributes to TE redistribution from the sediment into the pelagic phase thus increasing its bioavailability. It moreover contributes to the understanding of the specificity of this process depending on the water solubility or colloidal association of the TE.

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

4.2 TE content in animal's body

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

For both, amphipods and mussels, the whole TE content increased with the number of species in the mesocosms. TE affected by the experimental conditions were however not the same. In mesocosms with amphipods and mussels, the water soluble TE increased in amphipods which could be explained by the filtering activity of mussels, clearing the TE from the water column but concentrated them with their faeces near the sediment (Vaughn and Hakenkamp, 2001) where they are consumed by amphipods (Gergs and Rothaupt, 2008). In those mesocosms, for mussels the soluble TE content decreased whereas colloidal TE content increased, according to its increase in the water caused by amphipods' bioturbation. Finally for both, amphipods and mussels, the increase in TE content were more important in the presence of tubificids. The tubificids' bioturbation activity might have transferred TE from deeper layers of the sediment to the pelagic zone (He et al., 2019; Mermillod-Blondin et al., 2007; Reible et al., 1996), or modified physical properties of sediment, e.g. redox potential (Graf and Rosenberg, 1997), increasing their bioavailability. Moreover, for the amphipods increase could have been via biomagnification (Mermillod-Blondin et al., 2004). Our study confirms the increase of TE transport over the sediment water interface by bioturbation (Remaili et al., 2016) and moreover demonstrates how increasing interactions between invertebrates affect TE accumulation depending on species and TE partitioning between colloidal and dissolved.

4.3 Body stores

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

The correlation between protein and triglyceride contents with number of species exposed lead to a significant increase of WEBB in amphipods whereas it declined in mussels. This pattern highlights better experimental conditions for amphipods as they benefitted from the presence of tubificid preys (Mermillod-Blondin et al., 2004) and hence increased their energy. Amphipods might also have benefitted from mussels which concentrate the food items (e.g. bacteria) in the surface of the sediment (Vaughn and Hakenkamp, 2001) or produce consumable feces (Gergs and Rothaupt, 2008). In turn, physical interactions between amphipods and mussels might have disturbed the filtration rate of mussels and lead to the observed decrease in their triglyceride content (and the low change in TE concentration). The pattern observed for glycogen in amphipods is more complex with a strong decrease in mesocosms with amphipods and mussels together. This might be due to the increase of certain TE in the water of these mesocosms, especially for the toxic ones such as As, Cd, Cu, and Zn (Borgmann et al., 2005). Glycogen is used by amphipods to cope with harsh environmental conditions and may strongly decrease in presence of toxic compounds (Dehedin et al., 2013; Maazouzi et al., 2011; Marmonier et al., 2013). The physiological state of the organisms in terms of their energetic stores was not that strongly impaired by the TE during the short exposure time (7 days) and the relatively low TE concentrations in the sediment. Nevertheless, some of the TE were negatively correlated with body stores of amphipods (Fe, Ga, almost all REE, Sc, Th, V, and Y) and mussels (B, Sb, Sc and Th). However, further studies are needed to understand the underlying mechanisms and the effects of TE over a longer term.

5 Conclusion

Our study confirmed the strong effect of bioturbation of sediments by aquatic invertebrates on the transfer of colloidal and organic matter associated TE to the pelagic compartment, whereas the soluble fraction of TE was not affected. We also highlighted that interspecific interactions appear to be a key driver explaining both the resuspension of TE, depending on their characteristic, and the exposure/contamination of species through another species activity. Previous studies combining several species with different trophic positions focused on the biomagnification of TE along the food web, but our study revealed that a combination of several species would also control indirect contamination via increased bioavailability of TE by bioturbation activities. Moreover, we could not demonstrate high energy allocation in organisms exposed to TE but amphipods however, clearly benefitted from the additional energetic resources provided by tubificids. Further studies are required to measure the intensity of these interactions with more and or other functional feeding groups.

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Figure legends

Figure 1. Mean (± SD) concentrations of nitrate (NO_3), phosphate (PO_4) and ammonium (NH_4) in water according to the five experimental conditions. Significant differences are indicated by letters and numbers.

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

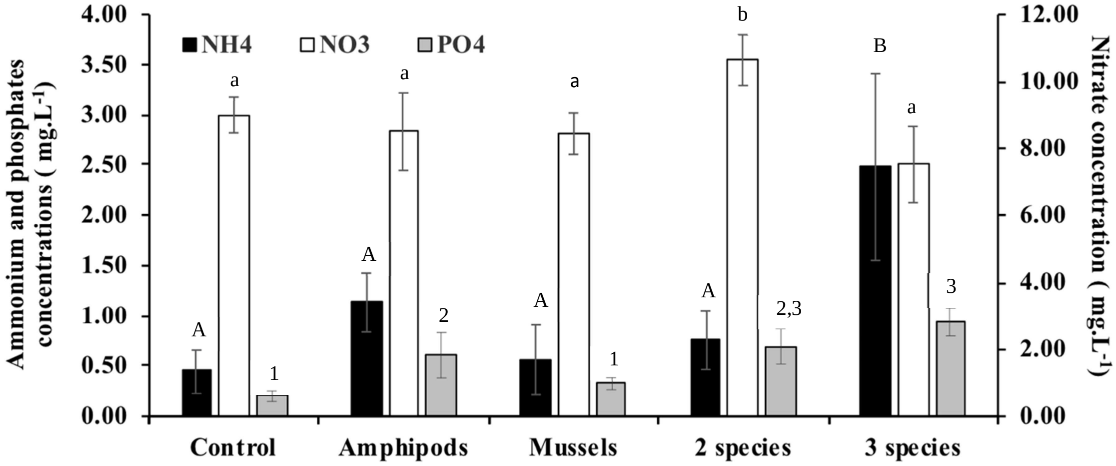
Figure 3. Mean (± SE) of selected TE concentrations in water according to the five experimental conditions: (a) total content in Rare Earth Elements (REE), (b) iron concentration, (c) Sum of Cr, U and Pb, (d) total content in highly Soluble Trace Element (STE) including (Li, B, Ba, Ca, K, Mg, Mn, and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

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

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

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

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



703

704 **Figure 1.**

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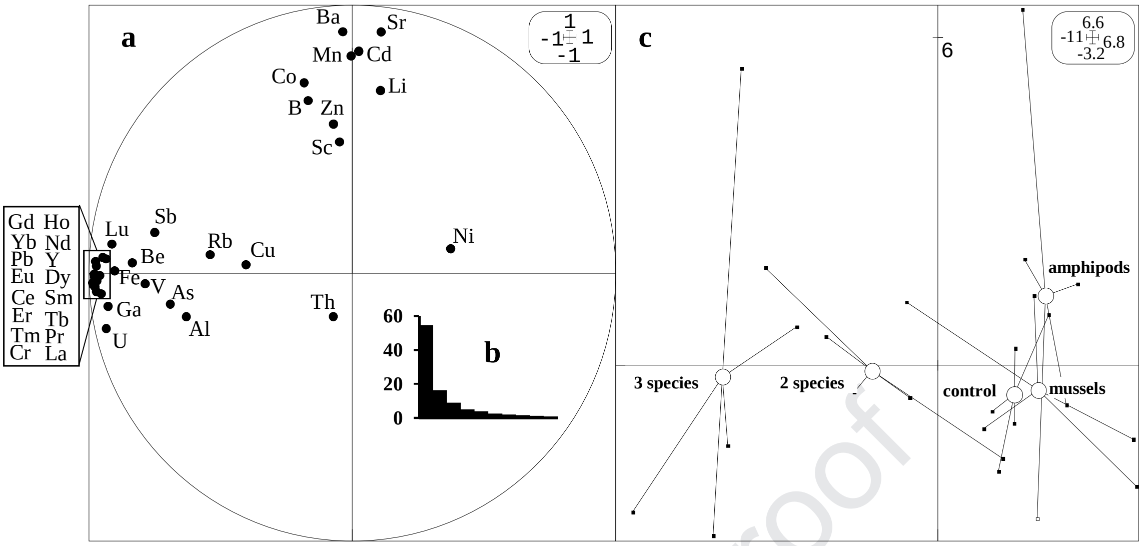
706 

707 **Figure 2.**

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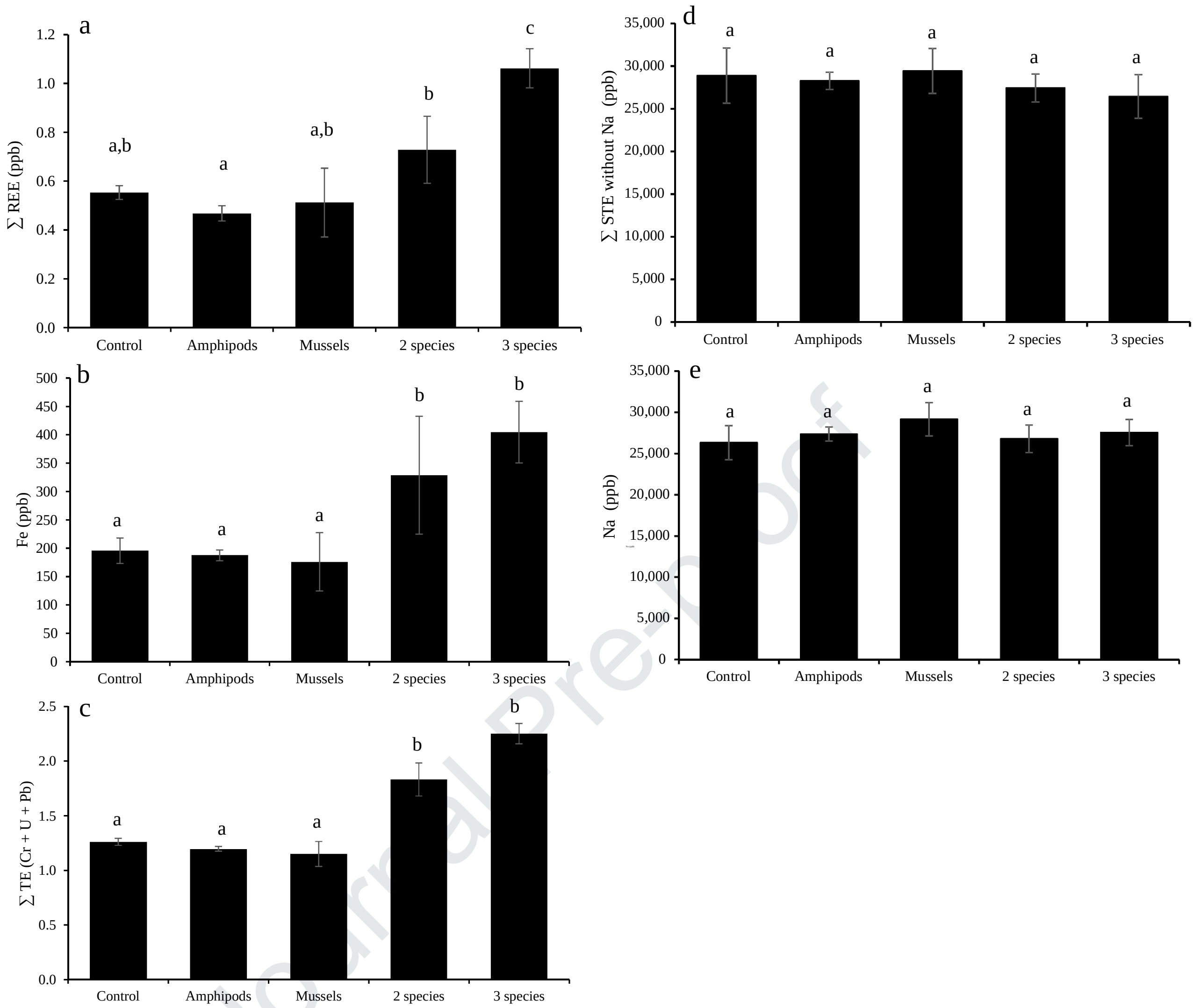
a
∑ REE (ppb)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
a,b
a
a,b
b
c
Control
Amphipods
Mussels
2 species
3 species
b
Fe (ppb)
0
50
100
150
200
250
300
350
400
450
500
a
a
a
b
b
Control
Amphipods
Mussels
2 species
3 species
c
∑ TE (Cr + U + Pb)
0.0
0.5
1.0
1.5
2.0
2.5
a
a
a
b
b
Control
Amphipods
Mussels
2 species
3 species
d
∑ STE without Na (ppb)
0
5,000
10,000
15,000
20,000
25,000
30,000
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a
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Control
Amphipods
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2 species
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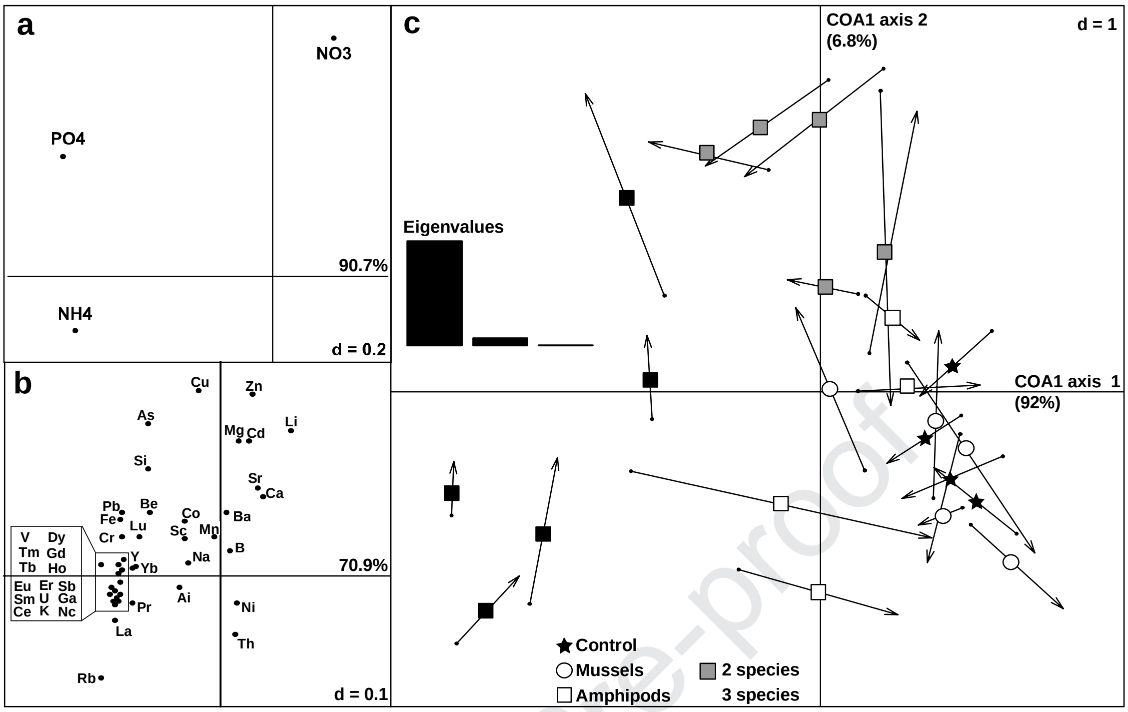
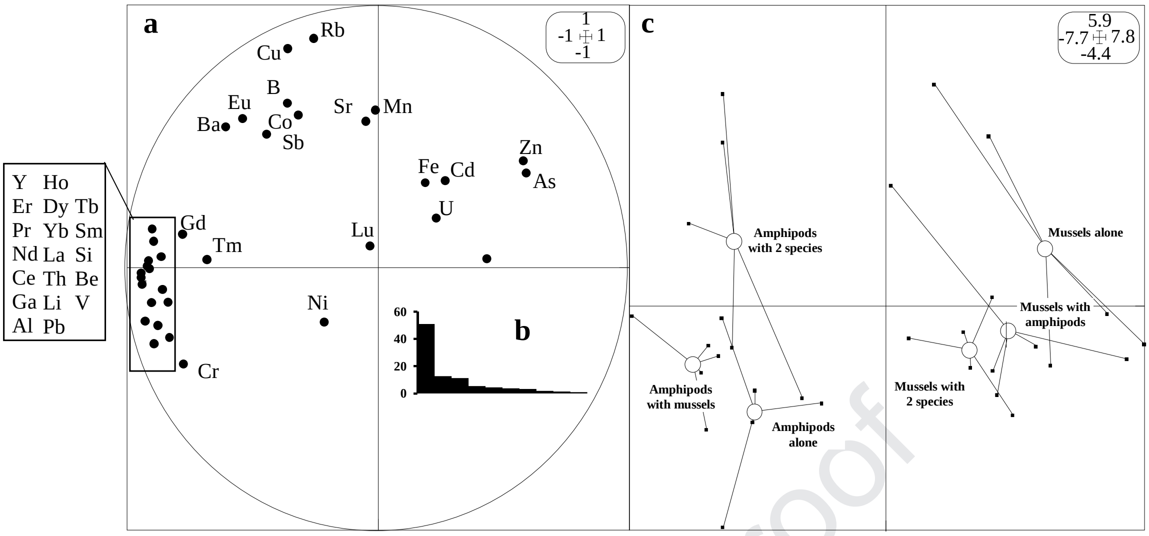
Control
Amphipods
Mussels
2 species
3 species

Figure 3.

727 

728 **Figure 4.**

729



730

731 **Figure 5.**

732

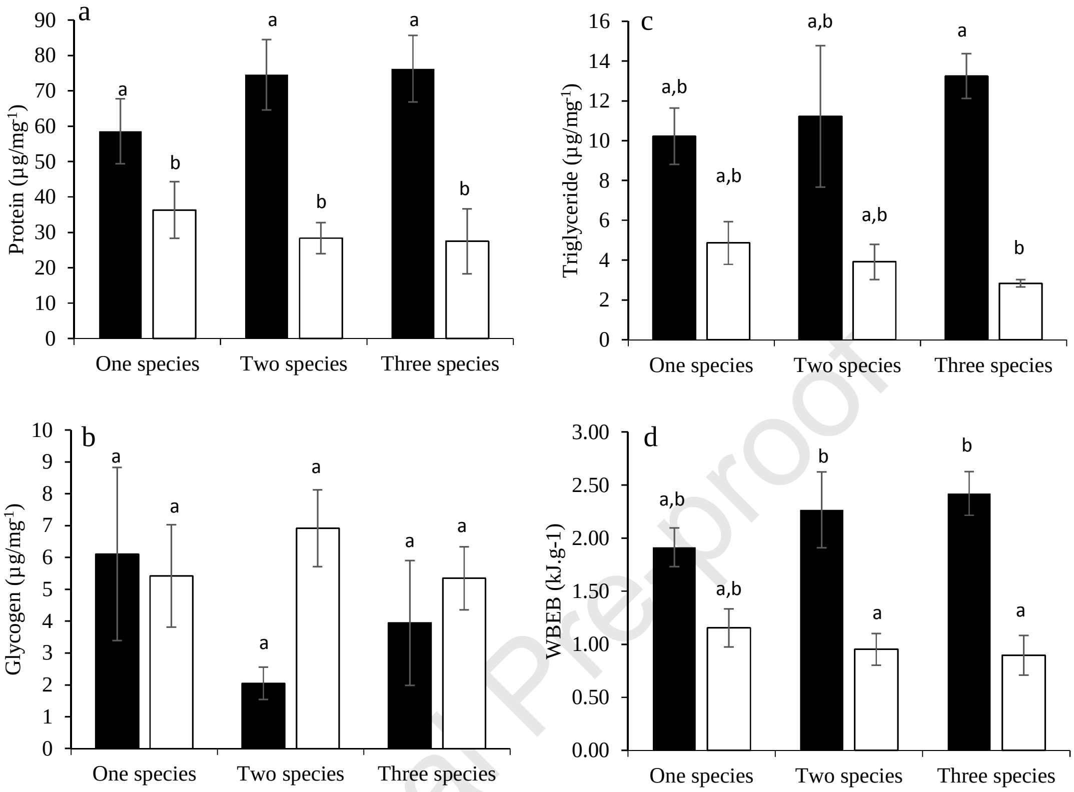

a
Protein (µg/mg-1)
90
80
70
60
50
40
30
20
10
0
One species
Two species
Three species
c
Triglyceride (µg/mg-1)
16
14
12
10
8
6
4
2
0
a,b
b
b
Glycogen (µg/mg-1)
d
WBEB (kJ.g-1)
3.00
2.50
2.00
1.50
1.00
0.50
0.00

Figure 6.

Highlights

- Macroinvertebrates bioturbation affects trace elements fate among sediment and water
- Trace elements bioturbation depends on their solubility or colloidal association
- Trace elements bioturbation depends on macroinvertebrates biological traits
- Interspecific interactions are key drivers for trace elements bioaccumulation
- 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: