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# Invertebrate grazers affect metal/metalloid fixation during litter decomposition

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## HIGHLIGHTS

- Invertebrate grazer enhance the fixation of most elements into organic sediments.
- Invertebrate grazers enhance the mobilization of DOC and nitrogen.
- Higher trophic level control elemental fixation/remobilization.

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## ABSTRACT

Plant litter and organic sediments are main sinks for metals and metalloids in aquatic ecosystems. The effect of invertebrates as key species in aquatic litter decomposition on metal/metalloid fixation by organic matter is described only for shredders, but for grazers as another important animal group less is known. Consequently, a laboratory batch experiment was conducted to examine the effect of invertebrate grazers (*Lymnaea stagnalis* L.) on metal/metalloid fixation/remobilization during aquatic litter decomposition. It could be shown that invertebrate grazers facilitate significantly the formation of smaller sizes of particulate organic matter (POM), as shown previously for invertebrate shredders. The metal/metalloid binding capacity of these smaller particles of POM is higher compared to leaf litter residuals. But element enrichment is not as high as shown previously for the effect by invertebrate shredders. Invertebrate grazers enhance also the mobilization of selected elements to the water, in the range also proven for invertebrate shredders but different for the different elements. Nonetheless invertebrate grazers activity during aquatic litter decomposition leads to a metal/metalloid fixation into leaf litter as part of sediment organic matter. Hence, the effect of invertebrate grazers on metal/metalloid fixation/remobilization contrasts partly with former assessments revealing the possibility of an enhanced metal/metalloid fixation.

## 1. Introduction

Enhanced concentrations of metals/metalloids in freshwater and aquatic sediments are a global problem for several types of freshwater ecosystems (Kouba et al., 2010; Schaller et al., 2013). This results in an enhanced environmental risk for associated ecosystems and organisms especially with concentrations of metals/metalloids becoming toxic to many species and thereby affecting many processes within these ecosystems (Nriagu and Pacyna, 1988). The metals/metalloids are transported by running water as cations, inorganic complexes and/or organic complexes of humic/fulvic acids as part of dissolved organic carbon (DOC) (Christensen et al., 1999; Alberic et al., 2000). Some part of metals is also transported in association with suspended particles. Element complexes may themselves adsorb on organic and inorganic particles, leading to a deposit in sediments (Sridhar et al., 2008), particularly in slowly flowing or standing water, like wetlands, pools in streams and lakes.

Leaf litter but also other plant litter is the most important energy source in e.g. littoral zones, wetlands or the krenal (spring region) and rhitral (stream) region of running water ecosystems being allochthonous systems. Leaf litter, settled on the bottom of the water body, will eventually be decomposed. Leaf litter decomposition proceeds in three distinct temporal stages of leaching, conditioning and fragmentation (Gessner et al., 1999). During primary decomposition by microorganisms, DOC emerges from the litter and microorganisms and their exudates form a heterotrophic

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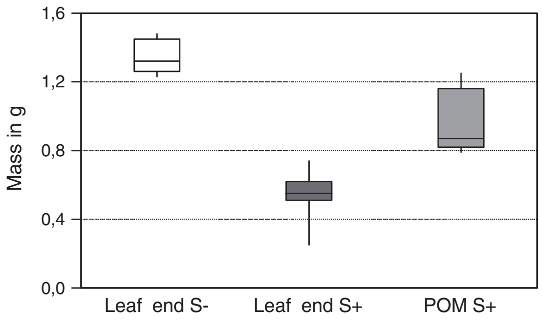

**Fig. 1.** Remaining mass of different fractions of organic matter of the different treatments with (S+) and without (S−) invertebrate grazers (*Lymnaea stagnalis* L.) at the end of experiment. Significant differences ($p < 0.001$) were found between leaf material of the different treatments (S+/S−) and between leaf material and smaller sizes of organic matter (2000–63 μm, POM) for treatment with invertebrate grazers ($p < 0.01$). The amount of POM at the end of experiment in treatment without invertebrate grazers was zero.

biofilm (Kominkova et al., 2000). Microorganisms involved in biofilm formation like bacteria and fungi are known to accumulate high amounts of metals/metalloids. Beyond that, the accumulation of elements depends on exudates (Huang et al., 2000), which also influences the survival of the biofilm (Pirog, 1997). While the primary decomposition proceeds, the leaf litter serves as main food source for invertebrates (Leroy and Marks, 2006). A well known key species in aquatic litter decomposition is *Gammarus pulex* L., (a member of the functional group of shredders) (belonging to the group of invertebrate shredders) (Graça, 2001). These invertebrates cut the leaves into smaller pieces of particulate organic matter (POM). During this process the accumulation potential of the resulting organic matter significantly increases (Schaller et al., 2010a,b). Another important species in litter decomposition is *Lymnaea stagnalis* L., a member of the functional group of grazers (Brady and Turner, 2010). These invertebrates literally graze on the organic structures and thereby harvesting biofilm. An effect of invertebrate grazers on metal/metalloid accumulation/remobilization during aquatic litter decomposition was postulated by Schaller et al. (2011) to be an increased remobilization, but has not been validated since then.

Therefore, an experiment was conducted to examine the effect of invertebrate grazers (*L. stagnalis* L.) on metal/metalloid fixation/remobilization during aquatic litter decomposition. To estimate the effects of invertebrate grazers for a wide range of element properties we measured magnesium and strontium (alkaline earth metal) as well as redox sensitive elements (manganese, cobalt, iron and uranium, whereas uranium has a reverse redox chemistry compared to the other).

## 2. Material and methods

### 2.1. Experimental setup

In the experimental setup 15 vessels (5 L buckets) each containing 5 g sand were used together with 3 L (liter) of soft water from a stream located in Tharandt (Germany). The sand was washed with HCl and NaOH prior to its use in the experiment to reduce the metal/metalloid content as well as the amount of soluble silica. A thorough rinsing with pure water until acid and hydroxide removal was applied thereafter. Leaf litter of *Alnus glutinosa* L. with high metal/metalloid content and a carbon/nitrogen ratio of 15 were added to 10 of the 15 vessels. Five of these 10 vessels containing sand and leaf litter were randomly selected and five large specimens (~4 cm body size) of the snail *L. stagnalis* L. were added to each vessel. Altogether three treatments were used in the experiment: 5 vessels containing 3 L of water and 5 g of sand (treatment sand), 5 vessels containing 3 L water, 5 g sand and 10 g leaf litter (~1.5 g dry mass), without snails (treatment S−) and 5 vessels containing 3 L water, 5 g sand and 10 g leaf litter (~1.5 g dry mass), additionally containing five snails (treatment S+). The leaf litter used in the experiment was fresh fallen leaves from *A. glutinosa* L. collected from uncontaminated plants at a plot in Tharandt near Dresden (Germany). The leaf litter was incubated into a stream affected by former uranium mining with a high metal/metalloid load near Neuensalz-Mechelgrün (Saxony, Germany) for two weeks using litter bags (nylon gaze of 63 μm mesh size). The conductivity of the water ranged between 1600 and 2070 $\mu S\,cm^{-1}$ at a pH range between 7.5 and 7.6 and Eh of 230 mV. The temperature of the water was relatively stable between 12.9 and 13.6 °C, because the water source is 150 m away from the experimental site and the spring-temperature is nearly 14 °C around the year. The element water concentration during the two weeks of exposure was in μg per liter: 46 000 (magnesium), 214 (iron), 1150 (manganese), 2.5 (cobalt), 35 (arsenic), 520 (strontium) and 175 (uranium). The exposure of leaf litter was done to allow microbial colonization of the leaf litter with microbes adapted to heavy metals as well as to allow the organic matter to accumulate the heavy metals. At this time, the microbes started the decomposition of leaf litter. The resulting heavy metal accumulation in leaf litter after the two weeks of pre-treatment were estimated applying random sampling of the leaf litter after transfer to the laboratory, and after homogenization.

The size of the individuals of *L. stagnalis* L. did not differ between the treatments, $p = 0.95$). Snail density was looked for to be in a natural range (Yurlova et al., 2006). For acclimation to the experimental conditions, the individuals of *L. stagnalis* were precultured for 5 d in the laboratory (in 10 L of soft water, 20 °C) before starting the experiment. During the pre-culture period, they were supplied with uncontaminated alder leaves. The test vessels

**Table 1**
Element content ($mg\,kg^{-1}\,DM^{-1}$) of leaves and particulate organic matter (2000–63 μm, POM) as different fractions of organic material at start and end of experiment. Significant differences were found between leaves at start and end for treatments without invertebrate grazers (S−) for Mg and Sr (all $p < 0.001$) and As ($p < 0.05$). Furthermore, significant differences were found between leaves at start and end for treatments with invertebrate grazers (S+) for Mg, Fe, Mn, Co, As and Sr (all $p < 0.001$) and U ($p < 0.01$). Significant differences were also found at end of experiment between leaves and POM for treatment with invertebrate grazer (S+) for Fe and As (both $p < 0.001$) and for Mn, Co and U (all $p < 0.05$).

| | | Mg | Fe | Mn | Co | As | Sr | U |
|---|---|---|---|---|---|---|---|---|
| Leaf start | Mean | 1872 | 2855 | 2684 | 9.11 | 23.3 | 94.8 | 469 |
| | SD | 81 | 364 | 398 | 1.30 | 3.9 | 3.6 | 52 |
| Leaf end S− | Mean | 974 | 2669 | 2331 | 8.08 | 17.1 | 55.1 | 453 |
| | SD | 44 | 355 | 405 | 0.75 | 2.3 | 3.0 | 64 |
| Leaf end S+ | Mean | 1164 | 732 | 1114 | 4.41 | 4.98 | 59.6 | 359 |
| | SD | 104 | 121 | 103 | 0.16 | 0.62 | 3.7 | 42 |
| POM end S+ | Mean | 1214 | 2760 | 2153 | 10.1 | 15.9 | 56.3 | 535 |
| | SD | 258 | 641 | 817 | 3.09 | 5.06 | 12.5 | 127 |