Shedding light on detritus: Interactions between invertebrates, bacteria and substrates in benthic habitats

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Chapter 3

Effects of copper on invertebrate-substrate interactions

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Abstract: Toxic pressure may alter bioturbation activities of aquatic invertebrates, thereby potentially affecting the links between invertebrate community structure and ecosystem functioning. This study therefore aimed to evaluate how toxicants affect invertebrate bioturbation and decomposition. To this purpose, the effects of copper on functionally distinct macrofaunal species (*Asellus aquaticus* and *Tubifex* spp.), decomposition (DOC) and microbial activity (AMR) and metabolic diversity (CMD) were determined in 5–day microcosm experiments. Invertebrate bioturbation was assessed with redox potential (Eh) profiles within the sediment. Concentration-response curves of the functional parameters DOC, AMR and CMD in the presence of *Tubifex* spp. were similar to the concentration-response curve of *Tubifex* spp. survival and resulted in comparable EC_{50} values. In contrast, the EC_{50} values of the functional parameters DOC, AMR and CMD in the presence of *A. aquaticus* are significantly lower than the EC_{50} of *A. aquaticus* survival. Sediment redox potential profiles showed that this was caused by a reduced interaction between *A. aquaticus* and the sediment at sub-lethal copper concentations. This points to a decoupling of invertebrate community structure and ecosystem functioning under stress, suggesting that functional parameters (e.g. decomposition), compared to structural paramaters, may serve as more sensitive and reliable parameters for assessing ecological water quality and ecosystem functioning.

***Keywords**: Copper, Bioturbation, Decomposition, Ecosystem Functioning, Sediment, Invertebrates.*

Decomposition of detritus is a vital ecosystem process driven by both microorganisms and invertebrate detritivores (Webster and Benfield 1986, Graça 2001, Gessner et al. 2010, Tank et al. 2010). Ecosystems are, however, under continuous toxic pressure, directly affecting decomposer organisms and therewith indirectly the ecosystem process they provide. However, to which extend toxic pressure on decomposing organisms cascades toward disordering ecosystem processes remains largely unknown.

Since a large part (>50%) of the detritus becomes trapped in subsurface sediments (Herbst 1980, Metzler and Smock 1990), the effect of bioturbation by invertebrates on organic matter processing gained increased interest (Mermillod-Blondin and Rosenberg 2006, Nogaro et al. 2009). It has indeed been demonstrated that detritus processing is largely influenced by invertebrate consumption and bioturbation activities that promote microbial decomposition by altering sediment texture, distributing solid particles, and introducing O_2 into otherwise anoxic zones (Meysman et al. 2006, Navel et al. 2010, Hunting and Van der Geest, 2011). Consequently, decomposition is directly related to the functional composition of invertebrate communities (François et al. 1997, Gérino et al. 2003, Mermillod-Blondin 2011, Hunting et al., 2012). However, toxic pressure may alter the locomotion and behavior of aquatic invertebrates (e.g. Van der Geest et al., 1999; Maltby et al., 2002; Brooks et al., 2011), potentially affecting these links between invertebrate community structure and ecosystem functioning. Therefore, the aim of the present study was to evaluate how toxicants affect the functional links between invertebrate bioturbation and ecosystem functioning. To this purpose, the effects of the model toxicant copper on functionally distinct macrofauna species (*Asellus aquaticus* and *Tubifex* spp.), detritus processing and microbial activity and metabolic diversity were determined in 5–day microcosm experiments. Altered locomotion and activity and reduced bioturbation were assessed with spatio-temporal redox (Eh) profiles within the upper sediment layer (Hunting et al., 2012).

Methods

Test organisms and test compound

Two aquatic invertebrate species were chosen to represent distinct types of sediment reworking. The oligochaete *Tubifex* spp. is an upward conveyor, i.e., deposit feeders that create burrowing networks in the sediment and defecate on the sediment surface. The isopod *Asellus aquaticus* is an omnivorous sediment dweller that acts as biodiffusor, i.e., grazing the upper layer of detritus and biofilms on sediment particles. *Tubifex* spp. was obtained from a laboratory culture. *Asellus aquaticus* was collected from nearby pristine ponds. Copper, an essential metal and common pollutant, was used as a model chemical stressor.

Microcosm preparation and sediment spiking

We constructed microcosms from 50-mL glass vials (25-mm diameter) filled with fine-grained, ignited quartz sand as mineral substrate (17 g/vial, grain size: 0.1–0.5 mm, sediment depth: 18 mm). The sediment was spiked with the following nominal copper concentrations (CuCl2.2H2O, copper standard, Fluka): 0 (control), 10, 20, 50, 100, 200, and 500 mg/kg dry weight. Appropriate amounts of copper stock solution were added to 420 g wet sediment in 1-L glass bottles. Treatments that required less or no metal stock solution were supplemented with deionized water, so equal volumes were added to all treatments. Freeze-dried, ground, and sieved stinging nettle (*Urtica dioica* L., ≤500 mm particle size, 365 mg) was added as detritus. The bottle was placed for 24 h on a roller bank (20 rpm) in order to homogenize the food-metal-sediment mixture, after which it was divided over the replicate (n=7 per treatment) microcosms (17 g/microcosm). Microcosms were carefully topped up with 35 mL of Dutch Standard Water (deionized water with 200 mg/L $CaCl_2*2H_2O$, 180 mg/L $MgSO_4*H_2O$, 100 mg/L $NaHCO_3$, and 20 mg/L $KHCO_3$; hardness is 210 mg as $CaCO_3$/L and pH 8.2). After settling of the sediment, microcosms were gently aerated and conditioned for one week, allowing copper to equilibrate with the sediment and a stable sediment layer to be formed.

Toxicity test

To evaluate the influence of copper on invertebrate survival, a five-day toxicity experiment was performed. Microcosms were kept at. 20 ± 1 °C and were constantly aerated. The experiment consisted of three invertebrate treatments per copper range: **1)** a control treatment without invertebrates; **2)** microcosms with 2 specimens of *A. aquaticus* and **3)** microcosms with 8 specimens of *Tubifex* spp., thereby containing equal invertebrate dry mass for the invertebrate treatments (Hunting et al., 2012). Each invertebrate treatment – copper concentration combination was replicated seven times. The experiments were initiated by introducing the invertebrates to the microcosms. At the end of the experiment (day 5), the sediment was sieved through a 350 µm sieve and the surviving invertebrates were counted, and the functional parameters detritus processing, bacterial activity and metabolic diversity, and development of Eh were determined. Detritus processing was measured as the concentration of dissolved organic matter in the overlying water visible as UV absorbance. To this purpose, the absorbance at 280 nm in the overlying water was measured at the end of the experiment.

We assessed bacterial activity and metabolic diversity in the sediment by community level physiological profiling (CLPP) using Biolog® GN microplates containing 95 unique single substrates (Biolog, Inc., Hayward, USA) (Garland and Mills, 1991). At the end of the experiment, pore water was sampled by pipetting 1 mL of sediment top layer, while preventing sampling of the overlying water. Samples were subsequently diluted 50x with DSW, and distributed over the 96 Biolog® GN wells. Plates were incubated for 48h and utilization patterns of 95 different single carbon sources were used to calculate the bacterial activity (average metabolic response, AMR) and community metabolic diversity (CMD) community (Garland, 1997).

We measured effects of copper on invertebrate locomotion and bioturbation as vertical sediment profiles of Eh recorded over time as described previously (Hunting et al., 2012). We recorded Eh with permanently installed redox microelectrodes and a calomel reference electrode connected to a Hypnos data logger (MVH Consult, Leiden, The Netherlands), both of which are newly developed in our laboratory (Vorenhout et al. 2011). We constructed Eh microelectrodes from Au-plated printed circuit board and placed them permanently in the middle of the sediment cores to allow high-resolution measurement of Eh in subsurface sediments (each mm [0–9-mm] depth, 2-mm width, every 15 min) throughout the experiment. Before the start of the actual experiment, we monitored Eh values and repositioned electrodes to ensure similarity in positioning with respect to the sediment surface among replicates. We converted Eh values to standard H-electrode output by adding 245 mV.

Chemical analysis

Actual copper concentrations in the sediment at the end of the experiment were determined by digesting duplicate 130 mg oven-dried subsamples per treatment in 2mL of a 4:1 mixture of nitric acid (65% p.a.; Sigma-Aldrich) and hydrochloric acid (37% p.a., Sigma-Aldrich) in tightly closed Teflon bombs upon heating in an oven at 140 ^{o}C for 7 h. The digested samples were diluted with 8 mL of deionized water and allowed to settle overnight at 5^{o}C. Copper concentrations in the samples were determined by flame atomic absorption spectrophotometry (Perkin-Elmer AAnalyst 100, Germany). The certified reference material ISE 989 Riverclay (Wageningen Agricultural University, The Netherlands) was used for quality assurance. The measured metal test concentrations were corrected for recovery and used to calculate the actual copper concentrations in the sediment per treatment.

Data analysis

The LC50, i.e. the actual toxicant concentration in the sediment at which 50% mortality was observed compared to the control, was calculated according to the logistic response model adopted from Haanstra et al. The following equation, $y = c/(1+e^{b(\log(x)-\log(a))})$, was fitted through the concentration response data, in which *y* represents the effect parameter (survival); *x* the actual exposure concentration; *a* the EC_{50}; *b* the slope of the logistic curve; and *c* the average survival in the control.

To evaluate the impact of copper on the invertebrate-mediated functional parameters, we considered the produced DOC, and the AMR and CMD in the presence of invertebrates as relative to the control treatment without invertebrates, i.e. measurements in the control treatment were subtracted from the measurements in the invertebrate treatments (residuals). The EC_{50}, i.e. the actual copper concentration in the sediment at which 50% reduction of the functional parameters was observed compared to the control without copper, was calculated as described above.

To evaluate the impact of copper on invertebrates locomotion and bioturbation, we considered the increase of sediment redox potential in the presence of invertebrates as relative to the control treatment without invertebrates, i.e. measurements in the control treatment were subtracted from the measurements in the invertebrate treatments (residuals). Eh residuals at day 5 of the experiment were subsequently plotted over depth against the actual copper concentrations by linear interpolation (Hammer et al., 2001).

Results

The actual copper concentrations in the sediment were: 5.6 (control), 15.3, 21, 55.3, 105.9, 176.2 and 487.6 mg Cu/kg dw sediment. This ranged between 88-111% of the nominal values in the 20-500 mg Cu/kg dw sediment range, but was higher than the nominal values of the 0 and 10 mg Cu/kg dw sediment microcosms. This is conform expectation as copper is an essential element.

Considering the results of the treatments without invertebrates, DOC, bacterial activity (AMR) and metabolic diversity (CMD) showed a gradual decrease with increasing copper concentrations in the sediment (Fig. S1)[1], while Eh remained unaffected (Fig. S2)[1]. Results of the treatments containing the invertebrates *Tubifex* spp. and *A. aquaticus* are shown in figure 1a-h. All invertebrates in the control treatment without copper were still alive at the end of the experiment. Clear concentration response curves were observed for the effect of copper on invertebrate survival, and the functional parameters DOC, AMR and CMD. From these concentration-response curves, the actual EC_{50} values with their 95% confidence intervals

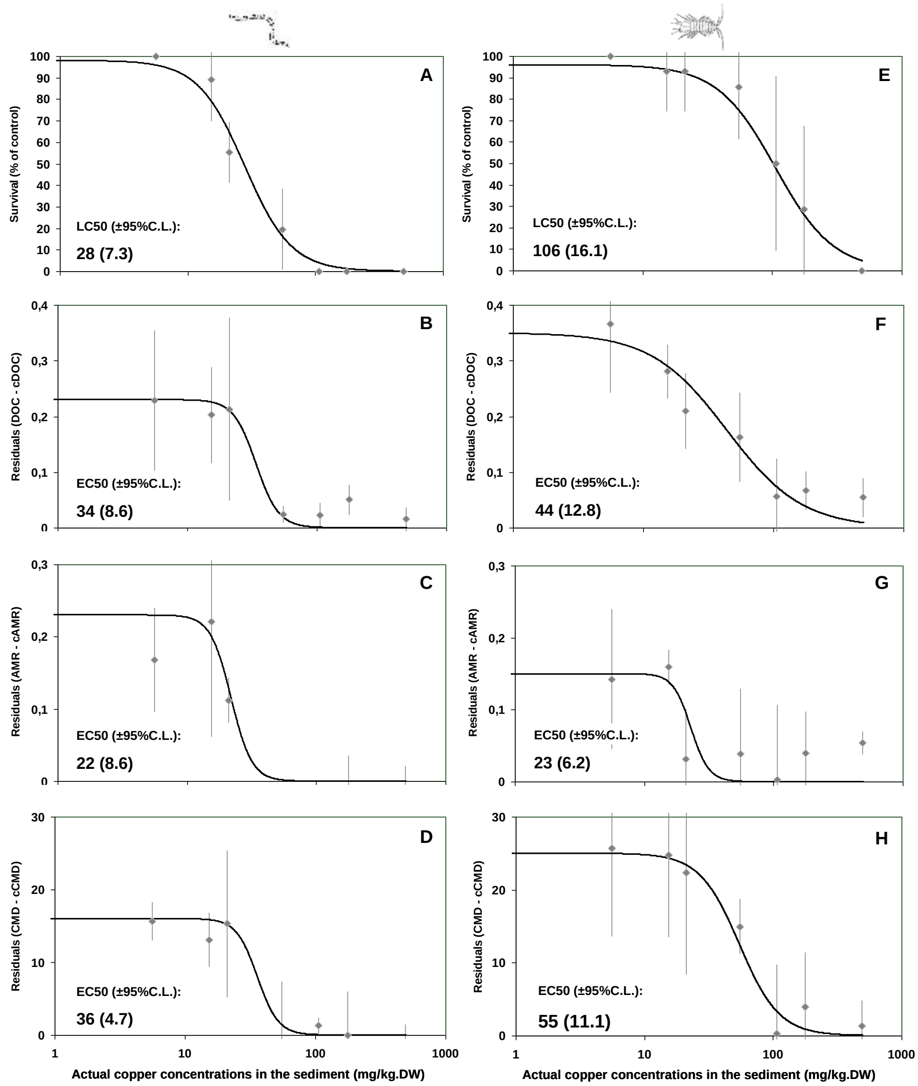

*Fig. 1: Concentration-response curves for the effect of copper (Cu) in the sediment after the 5-day experiment on (**A,E**) survival (% of control); and the residuals ([treatment measurements] - [measurements in controls without invertebrates] of (**B,F**) DOC concentrations in the overlying water; (**C,G**) Bacterial Community Metabolic Diversity (CMD); and (**D,H**) Average Metabolic Response (AMR) of the bacterial community. Provided are LC_{50}/EC_{50} with 95% C.L.*

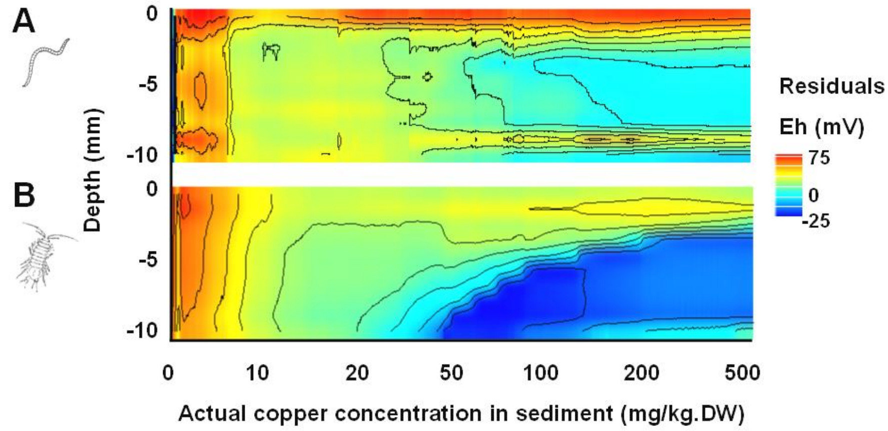

*Fig. 2: Sediment redox potential (mV) in the top-layer (0-9 mm depth) in the treatment containing the invertebrates (**A**) Tubifex spp. and (**B**) A. aquaticus as compared to the control treatments without invertebrates (Residuals, [treatment measurements] - [measurements in controls without invertebrates]).*

were calculated (Figure 1). Concentration-response curves of the functional parameters DOC, AMR and CMD in the presence of *Tubifex* spp. are similar to the concentration-response curve of *Tubifex* spp. survival and resulted in comparable EC_{50} values (Fig. 1 a-d). In contrast, the EC_{50} values of the functional parameters DOC, AMR and CMD in the presence of *A. aquaticus* are significantly lower than the EC_{50} of *A. aquaticus* survival (Fig. 1 e-h).

Development of sediment Eh profiles over time differed between microcosms with and without invertebrates and between invertebrate treatments. Eh residuals at day 5 (invertebrate treatment minus control treatment without invertebrates) are presented in figure 2a,b. *Tubifex* spp. mainly enhanced Eh at the top layer of the sediment and in deeper layers of the sediment only at lower copper levels (Fig. 2a). This positive effect rapidly decreased with increasing copper concentrations, although a slight increase in Eh was visible in deeper layers (8-9 mm depth). Since no *Tubifex* spp. survived these copper levels, this observed enhancement of Eh is probably the result of continuous irrigation of the created burrow-network. In the absence of copper, *A. aquaticus* homogenized the entire top layer (1 cm) of the sediment, but this positive effect on Eh rapidly decreased with increasing copper concentrations leaving the sediment completely stratified (Fig. 2b).

Discussion

The effect of toxic pressure on biotic interactions and ecosystem functioning remains poorly understood. Only a limited number of studies considered the effects of polluted leaf litter on invertebrates consumption (Brooks et al., 2009; Bundschuh et al., 2012), but the effect of toxicants on invertebrate-sediment interactions remains largely unknown. This study compared invertebrate survival and invertebrate mediated functional responses to sediment copper amendment and demonstrated that copper exposure resulted in reduced invertebrate bioturbation activities, microbial diversity and activity, and detritus processing, thereby illustrating that copper contamination significantly affects the link between invertebrate bioturbation and ecosystem functioning.

Bacterial metabolic diversity and metabolic activity also decreased with increasing copper concentrations in the absence of invertebrates. Bacterial conditioning of detritus is known to enhance invertebrate feeding of detritus (e.g. Graça et al. 2001, Canhoto and Graça 2008). It is thus possible that reduced decomposition was the result of reduced detrital palatability to invertebrates due to copper toxicity to the bacterial community, although bacterial conditioning typically becomes effective long after our experimental period (5-days) (e.g. Danger et al, 2012). Moreover, increasing evidence suggests that contaminated detritus has stronger impacts on invertebrates than on bacteria, and adversely affect invertebrate performances (Gonçalves et al. 2011). This is confirmed for a number of invertebrate species, showing that changes in feeding behavior is responsible for reduced decomposition rates (Macedo-Sousa et al. 2007, 2008, Pestana et al. 2007, Roussel et al. 2008). In the present study, the adverse effects of copper on decomposition thus relied on reduced invertebrate activity.

Invertebrate bioturbation typically enhances bacterial activity, detritus processing and sediment Eh (Solan et al., 2004; Mermillod-Blondin, 2011; Hunting et al., 2012), as observed in the present study. However, this positive effect decreased with increasing copper concentrations in the sediment. For *Tubifex* spp., the decrease in functional parameters coincided with the decrease in *Tubifex* spp. survival. In contrast, the decrease in functional parameters for *A. aquaticus* occurred at lower copper concentrations than the decrease in *A. aquaticus* survival. This is reflected by the sediment spatio-temporal redox profiles of the upper 10 mm of the sediment that visualized the interaction of invertebrates with the sediment. Bioturbation activities of *Tubifex* spp. and the dependent functional parameters clearly relied on the survival of *Tubifex* spp. In contrast, *A. aquaticus* revealed a decreased influence on sediment Eh while the invertebrates were still alive and concomitantly a reduced influence of the invertebrate on ecosystem functioning. This suggests that sub-lethal copper concentrations reduced the bioturbating activities of *A. aquaticus*, resulting in a decreased decomposition, resulting in a decoupling of invertebrate community structure and ecosystem functioning.

The observed differences between *Tubifex* spp. and *A. aquaticus* in the effect of copper on the link between invertebrate survival and functional parameters between were likely caused by differences in locomotion and feeding behavior, i.e. *Tubifex* spp. lives within the sediment, while *A. aquaticus* is crawling on top of the sediment. Since copper was spiked to the sediment, *A. aquaticus* was able to avoid direct exposure to toxicants, while *Tubifex* spp. typically remains within the sediment and was therefore continuously exposed to toxicants. Avoidance behavior has often been observed for *A. aquaticus* when exposed to a variety of toxicants (e.g. Blockwell et al., 1997; Bundschuh et., 2011). Although a number of physiological and behavioral responses to toxicants (including reduced locomotion) have been observed for oligocheates (e.g. O'Gara et al., 2004), these did not impact the *Tubifex* spp.-sediment interaction in this study. This study therefore demonstrates that the sensitivity of functional parameters depends on the feeding strategy and locomotion of the invertebrate species.

This study demonstrated that toxicants can affect the functional links between invertebrate bioturbation and ecosystem functioning, highlighting the importance of species specific feeding strategies and locomotion for the effects of chemical stressors on ecosystem functioning. Here we show a decoupling of invertebrate community structure and ecosystem functioning under chemical pressure, suggesting that functional parameters (e.g. decomposition) may serve as more sensitive and reliable parameters for assessing ecological water quality and ecosystem functioning.

[1]*Supplementary data related to this chapter can be found at:* http://dx.doi.org/10.1016/j.envpol.2013.05.027.