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

Hunting, E.R.

Publication date
2013

Chapter 6

Invertebrates as driver for decomposition, sediment mixing and bacterial communities:

An outdoor mesocosm experiment

Manuscript under review.
Hunting. E.R., A.A. Kampfraath, C. Mulder, M.H.S. Kraak, A.M. Breure and W. Admiraal. **Invertebrates as drivers of detritus processing, sediment mixing and bacterial communities: an outdoor mesocosm experiment.**

Abstract: Decomposition of organic matter is a central ecosystem process governed by microorganisms and invertebrates. Several parameters are at hand to quantify the decomposition process, but these are seldom studied in coherence and mainly rely on laboratory experiments. Therefore the aim of this study was to test the response of bacterial functional diversity and activity, biogenic mixing depth and detritus processing to activities of invertebrate species combinations in outdoor mesocosms. Metabolic diversity and activity of the bacterial communities did not differ between treatments, indicating that invertebrates did not influence bacterial activity. The biogenic mixing depth (BMD), represented by the depth until the average redox potential is enhanced relative to the control, increased with increasing number of invertebrate btioturbator types, rather than with increasing number of species. Detritus processing, measured as DECOTAB mass loss, was substantially higher in treatments containing invertebrates compared to the control treatment without invertebrates. For DECOTAB mass loss, the presence of shredders (the isopod *A. aquaticus* or the amphipod *G. pulex*) was of predominant importance, demonstrating the importance of functional identity rather than diversity. By studying several functional parameters in coherence, we demonstrated that different ecosystem processes responded differently to invertebrate species composition. We showed that the sentinel for decomposition (DECOTAB mass loss) reflected the functional composition of the invertebrate community, whereas the microbial parameters did not, despite clear-cut effects on biogenic mixing. It was indicated that solar radiation and its consequent effects on the variable redox conditions may decouple invertebrate-bacterial interactions.

Key words: *decomposition, aquatic invertebrates, bioturbation, functional traits, bacterial community structure, cellulose decomposition, redox potential.*

Decomposition of organic matter is a central process in ecosystem functioning and therefore considered a promising proxy to evaluate the health of ecosystems (e.g. Gessner et al. 2010; Kampfraath et al. 2012). Detritus processing is driven by invertebrate and microbial activities, in which processing rates are the result of direct consumption and invertebrate bioturbation activities that promote microbial decomposition (Covich et al. 2004, Nogaro et al. 2009, Hunting et al. 2012).

The significance of invertebrate species composition for detritus processing is typically evaluated with a number of functional parameters. Firstly, various studies have demonstrated that invertebrate activities promote bacterial activities, the stimulus depending on the type of invertebrate sediment reworking (Mermillod-Blondin and Rosenberg 2006; Nogaro et al. 2009). In accordance, microbial community structure was observed to be affected by bioturbation (Bertics and Ziebs 2009; Hunting et al. 2012). However, metabolic diversity or substrate utilization patterns, may be a more important attribute of ecosystem functioning than bacterial community structure (Salles et al. 2009; Gravel et al. 2011). Secondly, invertebrates have been shown to affect the geochemical characteristics of the sediment (Meysman 2006). This is often quantified by following the redistribution of chemical tracers such as luminophores (e.g. Mermillod-Blondin et al. 2002), digital imaging of sediment cross-sections (e.g. Solan et al. 2004) and profiling oxygen penetration (e.g. Birchenough et al. 2012), or redox potential (Hunting and Van der Geest 2011; Hunting et al. 2012). Thirdly, standardized substrates such as cotton strips or DECOTABs that are composed mainly of cellulose, a major constituent of plant litter, have been used to obtain measurements of the cellulose decomposition potential as a standardized surrogate measure reflecting litter decomposition (Boulton and Quinn 2000; Tiegs et al. 2008; Young et al. 2008; Imberger et al. 2010; Kampfraath et al. 2012). These three groups of functional parameters are rarely studied simultaneously, especially not in experiments, and therefore the relative importance, reliability and cohesion remain uncertain. Moreover, most of our understanding of invertebrate interactions with sediments and their effects on decomposition is based on laboratory single species experiments.

Experiments are thus required that test the predictive potential of functional metrics in invertebrate detrital food webs under (quasi) natural conditions. Therefore, our study aimed to test whether a simultaneous response of a number of functional parameters to activities of multispecies invertebrate assemblages could be derived from their responses to single invertebrate species experiments under laboratory conditions. To this purpose, bacterial functional diversity and activity, sediment redox potential and DECOTAB mass loss were measured in laboratory microcosms and outdoor mesocosms in the presence of single invertebrate

species and manipulated multi-species assemblages.

Methods

Mesocosms and test organisms

Mesocosms.— A 21-d mesocosm experiment was performed in June 2011. Our outdoor mesocosms consisted of rectangular 90 L plastic tubs (L*W*H, 66 x 34 x 30 cm, respectively). They contained ca. 40 L of rainwater and 18.5 L of sediment made of standardized garden soil (Baseline, Maxeda DIY, Diemen, The Netherlands) and quartz sand (0.1-0.5 mm; Dorsilit, Eurogrit, Papendrecht, The Netherlands) mixed in a ratio of 5 L soil per 25 kg sand. These mesocosms were placed in concrete containers filled with water to buffer temperature fluctuations. A gauze screen (mesh size 1 mm) was pulled over the concrete containers to reduce colonization by allochthonous fauna. Before the experiment started, the mesocosms were left for two days to allow the sediment to settle and the microbial communities present in the soil and rainwater to acclimate. During the experiment, the overlaying water was gently aerated with a permanently installed air compressor aeration system. Sediment temperature and solar radiation (pyranometer connected to a datalogger, CR 10X) were measured every 5 min.

Invertebrates.—We used 5 invertebrate species that represented 3 types of bioturbators as outlined by e.g. Nogaro et al. (2009). The isopod *Asellus aquaticus* and the amphipod *Gammarus pulex* are omnivorous sediment-dwellers that act as biodiffusors, i.e., grazing the upper layer of detritus and biofilms on sediment particles. Larvae of the nonbiting midge *Chironomus riparius* create ventilated U-shaped tubes, feed on surface sediment material, and are considered gallery diffusors. The oligochaetes *Tubifex* spp. and *Lumbriculus variegatus* are both upward conveyors, i.e., deposit feeders that create burrowing networks in the sediment and defecate on the sediment surface.

Experimental design

Interactions between species were evaluated by considering the responses of functional parameters to multispecies assemblages as relative to their response to invertebrate single species incubations. To unravel potential interactions between invertebrate species and bioturbation type, the following invertebrate combinations were tested in outdoor mesocosms: [*Tubifex* spp. and *A. aquaticus*], [*Tubifex* spp., *A. aquaticus* and *C. riparius*], and [*Tubifex* spp., *A. aquaticus*, *C. riparius*, *L. variegatus* and *G. pulex*]. A control treatment without invertebrates was included and each treatment was replicated 5 times. Invertebrates were added up to equal total quantities of 500 mg.DW/m^2. After 21-d, we evaluated the influence of invertebrate species composition on detritus processing

(DECOTAB mass loss), bacterial activity (AMR) and functional diversity (CMD), and Biogenic Mixing Depth (BMD) by monitoring the development of the sediment redox potential (Eh) as described below.

To evaluate whether responses of functional parameters to multispecies invertebrate assemblages can be predicted from single species incubation under laboratory conditions, single species were simultaneously incubated in the laboratory in order to obtain the responses of the selected functional parameters required to calculate the expected values in mixed species assemblages as described below. One representative of each bioturbation type (i.e., biodiffusor, upward conveyor, and gallery diffuser) was incubated for 3 weeks in 100 mL glass laboratory microcosms with the same substrate and invertebrate densities as applied in the outdoor mesocosms. Each treatments was replicates 5 times. The average responses in single species incubations were used to calculate an expected response to the mixed assemblages in the outdoor mesocosms as described below (*Data analysis*).

Functional parameters

Bacterial activity (AMR) and functional (metabolic) diversity (CMD).—Bacterial activity (AMR) and metabolic diversity (CMD) in the sediment was assessed 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 Dutch Standard Water (DSW; a standardized synthetic analog of common Dutch surface waters, containing: 200 mg $CaCl_2 \bullet 2H_2O$, 180 mg $MgSO_4 \bullet 7H_2O$, 100 mg $NaHCO_3$, and 20 mg $KHCO_3$/L demineralized H_2O; pH 8.1, hardness 210 mg/L $CaCO_3$, alkalinity 1.2 meq/L), 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).

Biogenic Mixing Depth (BMD).—Sediment redox potential (Eh) profiles were used to characterize invertebrate interactions within sediment as previously described (Hunting et al., 2012). We measured Eh in 3 replicates of all treatments. We recorded Eh with permanently installed redox potential microelectrodes (each mm [0–7-mm] depth, 2-mm width, every 15 min) and a calomel reference electrode connected to a Hypnos data logger (MVH consult, Leiden, the Netherlands) (Vorenhout et al. 2011). Biogenic mixing depth (BMD) *sensu* Solan et al. (2004) was subsequently determined by evaluating at which sediment depth an increase in the average Eh over time was visible as relative to the control mesocosms

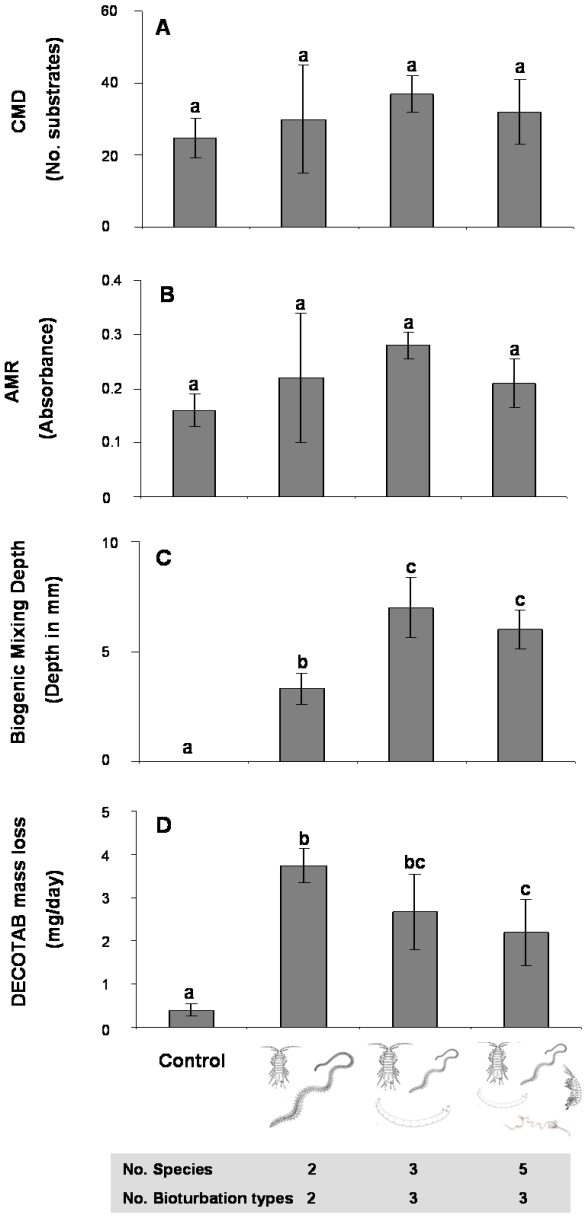

*Fig. 1. Responses of functional parameters (mean ± SD) to multispecies invertebrate assemblages compared to controls without invertebrates: (**A**) bacterial Community Metabolic Diversity (CMD); (**B**) Average Metabolic Response (AMR); (**C**) Biogenic Mixing Depth (BMD); (**D**) DECOTAB mass loss. Different letters indicate significant differences between treatments (one-way analysis of variance, Tukey's Honestly Significant Difference post hoc test, n = 5, p < 0.05). Number of species and bioturbator types are listed.*

without invertebrates.

Detritus Processing.—Detritus processing was measured as cellulose decomposition with cellulose-based decomposition and consumption tablets (DECOTABs) (Kampfraath et al. 2012). Each mesocosm received three DECOTABs (Ø 15 mm, h 5 mm, final volume 118 mm^3). DECOTABS were prepared from a mixture of 60 g cellulose (Sigma-Aldrich - #C6413), 20 g purified agar (OXOID Limited) and 60 µM ascorbic acid (Merck) per liter dH_2O After 21 days, the DECOTABs were removed and subsequently rinsed, dried (three days at 70°C) and weighed. This weight was subtracted from the original dry weight to calculate the daily DECOTAB mass loss.

Data analysis

Utilization patterns of the 95 carbon sources were analyzed using a Jaccard-based cluster analysis and one-way ANOSIM with subsequent Bonferroni corrected pair-wise comparison (Hammer et al. 2001). Expected bioturbation type-specific (Biodiffuser, Gallery diffuser and Upward conveyor) values for the selected functional parameters were derived from single species incubations. The observed consumption rates (DECOTAB mass loss) and microbial AMR and CMD values for each invertebrate assemblage in the mesocoms were compared with the functional parameter values that would be expected if no interactive effects were present (cf. Chapman et al. 1988; Blair et al. 1990; Wardle et al. 1997). Expected values for mixed invertebrate assemblages were calculated based on bioturbation type as the weighted average of their contribution to the invertebrate assemblage at the start of the mesocosm experiment. The residuals (Observed - Expected) were tested against the *null hypothesis* (no interaction) that the average residual equaled 0 (one sample T-test) (Hammer et al. 2001).

Results

Responses of the functional parameters are presented in Fig. 1A-D. All treatments showed a similar range of Bacterial Community Metabolic Diversity (CMD) and Average Metabolic Response (AMR), including the control without invertebrates (Fig. 1A,B). There was a considerable variability between replicates, and therefore the influence of invertebrates on bacterial community diversity and activity could therefore not be assessed. Cluster analysis of the metabolic diversity of the bacterial communities of the different treatments did not reveal any separation between the different treatments and again showed considerable variability between replicates, including the control (Fig. 2) (ANOSIM, Bonferroni-corrected pair-wise comparison: p = 0.88), indicating that potential influences of invertebrate assemblages on the functional composition of the bacterial community could not be detected (Fig. 2). We

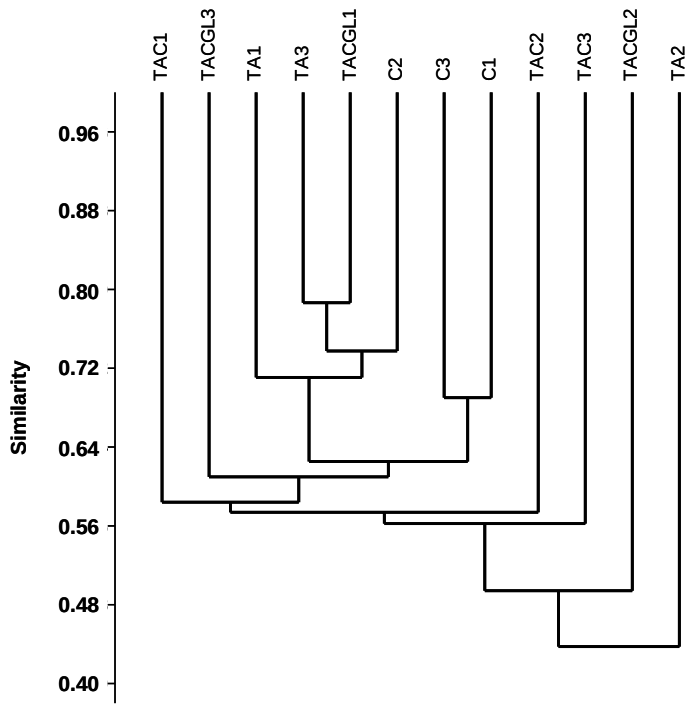

*Fig. 2. Jaccard based cluster analysis representing the level of similarity of the bacterial Community Metabolic Diversity (CMD) of the control (**C1-3**), and triplicate invertebrate containing treatments, including combinations of Tubifex spp. (**T**), Asellus aquaticus (**A**), Chironomus riparius (**C**), Lumbriculus variegates (**L**), and Gammarus pulex (**G**). The functional composition did not differ between treatments (one-way ANOSIM, p = 0.878).*

therefore did not compare expected and observed values for bacterial responses to the different invertebrate assemblages.

Spatiotemporal redox profiles revealed diurnal patterns that were coupled with solar radiation (Fig. 3), as Eh increased simultaneously with increasing solar intensity. The Biogenic Mixing Depth (BMD), represented by the depth until the average Eh is enhanced as relative to the control, differed depending on the combination of invertebrates (Fig. 2C), in which the invertebrate combinations [*Tubifex* spp., *A. aquaticus*, *C. riparius*] and [*Tubifex* spp., *A. aquaticus*, *C. riparius*, *L. variegatus* and *G. pulex*] revealed a significantly (One-Way ANOVA, Tukey-HSD, $p < 0.05$) enhanced BMD as compared to the invertebrate combination [*Tubifex* spp. and *A. aquaticus*]. The residuals (Observed values – Expected values derived from invertebrate monocultures, supplementary Fig. S1[1]) revealed that no significant interaction was visible in the [*Tubifex* spp. and *A. aquaticus*] treatments (Fig 4A). In contrast, residuals were significantly higher than 0 in the invertebrate treatments [*Tubifex* spp., *A. aquaticus* and *C. riparius*] and [*Tubifex* spp., *A. aquaticus*, *C. riparius*., *L. variegatus* and *G. pulex*], suggesting a significant enhancement of biogenic mixing compared to the single species incubations.

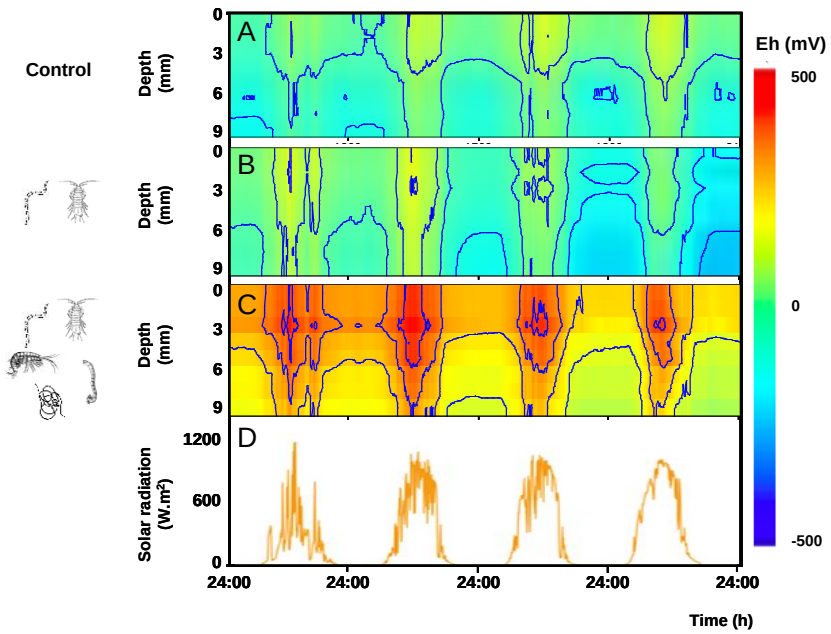

*Fig. 3. Examples of redox potential (Eh) profiles in depth (0-9 mm) and time (4 days) of the mesocosms containing **A)** no invertebrates; **B)** Tubifex spp. and Asellus aquaticus and **C)** Gammarus pulex, Asellus aquaticus, Chironomus riparius, Tubifex spp., and Lumbriculus variegates. Eh profiles show diurnal rhythms that correspond to **D)** solar radiation.*

Detritus processing, measured as DECOTAB mass loss, was substantially higher in the treatments containing invertebrates as compared the control treatment without invertebrates (Fig 2D), in which DECOTAB mass loss was significantly higher in the treatment containing [*Tubifex* spp. and *A. aquaticus*], as compared to the invertebrate combination [*Tubifex* spp., *A. aquaticus*, *C. riparius*, *L. variegatus* and *G. pulex*] (One-Way ANOVA, Tukey-HSD p = 0,008). The residuals (Observed - Expected) were significantly higher than 0 in the invertebrate treatment [*Tubifex* spp. and *A. aquaticus*], but were almost zero in the other invertebrate treatments (Fig. 4B), suggesting a significant enhanced DECOTAB mass loss in the [*Tubifex* spp. and *A. aquaticus*] combination as compared the single species incubations, while no interaction effects were observed in the other invertebrate treatments.

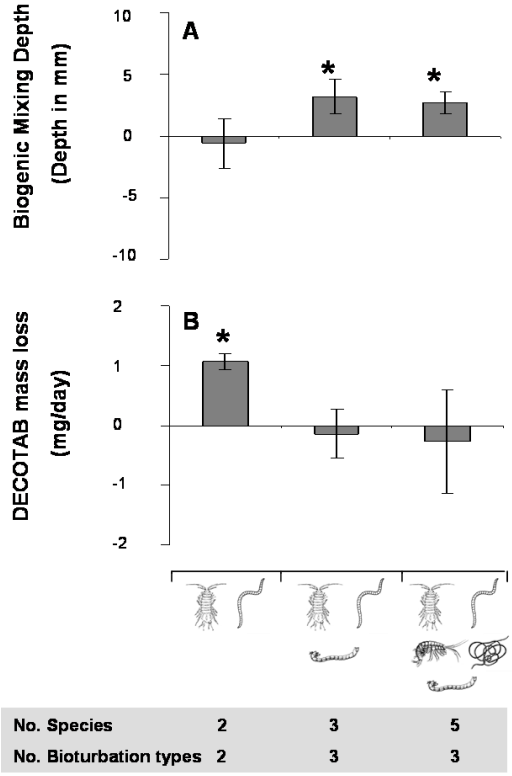

*Fig. 4. Residuals (observed – expected calculated from single species incubations) for Biogenic Mixing Depth (**A**) and DECOTAB mass loss (**B**), testing the null hypothesis that no interaction between invertebrate species occurred, i.e. residual equaled 0 (* indicates that residuals significantly deviates from 0, one sample T-test, n = 5, p < 0.05).*

Discussion

Previous studies that examined the effects of invertebrate activity on microbial (functional) diversity in laboratory microcosms (Mermillod Blondin and Rosenberg 2006; Navel et al. 2010; Bertics and Ziebis 2009) clearly revealed that sediment reworking by invertebrates shapes the composition of the benthic microbial community. However, in the present study, the functional bacterial composition (as represented by the number of substrates used) and the overall activity were variable and different treatments were not statistically separated. This suggests that the effects of sediment reworking on the functional composition of the microbial community were overruled by factors unrelated to invertebrate bioturbation. Several studies have demonstrated that solar radiation can have profound effects on both the structural and functional composition of the bacterial community due to e.g. detrimental effects on DNA (Denward et al., 1999; Piccini et al., 2009). It is likely that solar radiation overruled invertebrate-bacterial interactions in our mesocosms. Effects of solar radiation showed up to strongly impact diurnal changes of the sediment redox conditions in all mesocosms. Although the mechanisms underlying this observation remain uncertain, diurnal rhythms are typically visible in continuous redox potential measurements (e.g. Vorenhout et al., 2004, 2011). It has been demonstrated that redox condition is an important driver for bacterial community structure and activity (Bespalov et al., 1996; Bertics and Ziebis, 2009; Hunting and Van der Geest, 2011; Hunting and Kampfraath, 2013). Since sediment redox potential was similarly affected by solar radiation in all mesocosms, it is possible that a combined effect of solar radiation and subsequent changes in sediment redox potential was converging bacterial community structure in this study. Although this remains speculative and requires further examination, this outcome suggests that the frequently observed influences of invertebrates on bacterial diversity and activity in laboratory studies (Mermillod-Blondin, 2002; Nogaro et al., 2009; Hunting et al., 2012) can become less prominent in the presence of solar radiation, especially in shallow waters.

Sediment reworking by invertebrates resulted in elevated redox potentials within the upper layers of the sediment, reflecting an increased biogenic mixing depth (BMD) (Solan et al. 2004; Birchenough et al. 2012). Increasing diversity from 2 species to 3 species increased BMD, suggesting that increasing community complexity results in altered and wider distributions of the infaunal community and inherent biogeochemical characteristics of the sediment. The observed increase in biogenic mixing is very likely the result of infaunal organisms burrowing deeper in the sediment in response to epifaunal presence. However, increasing the number of species to 5, while maintaining the same number of bioturbation types, did not alter the BMD, suggesting that geochemical

characteristics of the sediment rely on functional diversity rather than species richness. Differences in invertebrate community structure are often visible in the geochemical characteristics of soft bottom sediments (e.g. Solan et al., 2004; Mermillod-Blondin and Rosenberg, 2006; Hunting et al., 2012). However, significant interaction effects were observed in our multispecies invertebrate mesocosms as compared to the single species incubations. BMD in multispecies assemblages tend to be higher than predicted from BMD's derived from single species incubations, suggesting that invertebrate species interactions have strong positive, yet unpredictable effects on the redox conditions of the sediment.

Cellulose decomposition, in the present study measured as DECOTAB mass loss, is increasingly used as a sentinel substitute of plant litter decomposition and to evaluate antropogenic impacts (pollution, habitat modification, etc.) on ecosystem functioning in natural environments (Gessner and Chauvet 2002; Tiegs et al. 2007; Young and Collier 2009; Imberger et al. 2010). The majority of studies concentrated on the use of commercially available cotton strips (e.g. Boulton and Quinn 2000 Young et al. 2008; Jenkins et al., 2013). Instead, we used the newly developed DECOTABs composed of 75% cellulose (Kampfraath et al. 2012). However, although it was demonstrated that DECOTABs were readily consumed by the shredding isopod *A. aquaticus* and by the collector-gatherer *C. riparius*, the extent to which they could be used to develop functional metrics for assessing biodiversity losses remained uncertain (Kampfraath et al., 2013). In this study, DECOTAB mass loss in the treatment containing 2 species revealed some overyielding, pointing to complementary mechanisms at very low levels of diversity. However, DECOTAB mass loss in multispecies assemblages containing 3 and 5 species could be predicted based on bioturbator-specific responses in invertebrate single species laboratory experiments, in which DECOTAB mass loss appeared to rely mainly on shredder (*A. aquaticus* and *G. pulex*) abundances. This suggests that biodiversity effects on ecosystem functioning were again relying on the functional composition rather than species richness. Although diversity effects of invertebrates and their activities on decomposition are often considered difficult to predict (e.g. Jonsson and Malmqvist 2000; McKie et al. 2008, 2009), DECOTAB decomposition was directly related to functional diversity in our mesocoms, especially at higher levels of diversity. This suggests that DECOTABs provide a promising tool to assess decomposition in relation to the (functional) composition of invertebrate communities.

Our study simultaneously evaluated functional metrics that reflect distinct components of the detrital food web in response to invertebrate community structure. Our results contribute to the notion that different ecosystem processes respond differently to changes in biodiversity (e.g. Woodward 2009; Reiss et al. 2009), in which the integrated process

(DECOTAB mass loss) seems more predictable than its separate components (bacterial activity and biogenic mixing). Testing functional parameters under natural conditions revealed that abiotic influences (e.g. solar radiation) can mask or even decouple invertebrate-bacterial interactions in the detrital foodweb.

[1]*Supplementary data related to this chapter will be available online*