Invertebrate footprints on detritus processing, bacterial community structure, and spatiotemporal redox profiles


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Invertebrate footprints on detritus processing, bacterial community structure, and spatiotemporal redox profiles

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Abstract. Detritus processing is driven by a complex interplay between macroinvertebrate and microbial activities. Bioturbation/feeding activities of invertebrates in sediments are known to influence decomposition rates. However, direct effects of invertebrates on bacterial communities and detritus processing remain ill-defined, mainly because identifying interactions between invertebrates and sediments is methodologically challenging. We incubated 5 macroinvertebrate species with various bioturbation/feeding traits separately in sediment-filled microcosms inoculated with bacterial communities for 5 d. At the end of the experiment, we assessed: 1) detritus processing (mass loss on ignition [LOI] and dissolved organic C accumulation in the overlying water [absorbance at 280 nm]), 2) bacterial community structure (intergenic spacer analysis [RISA]) and bacterial activity (electron transport system activity [ETSA]), and 3) development of redox potential (Eh) over time (with permanently installed microelectrodes). Invertebrates enhanced bacterial activity and detritus processing, and the magnitude depended on bioturbation/feeding traits. Bacterial community structure differed significantly between microcosms with burrowing invertebrates and microcosms with sediment-dwelling invertebrates. Eh profiles were similar among microcosms with invertebrates with similar bioturbation/feeding traits, but differed among microcosms with invertebrates with dissimilar bioturbation/feeding traits. Our results suggest that bioturbation by aquatic invertebrates mediates detritus processing, Eh dynamics, and structure of the microbial community. These findings highlight the significance of bioturbation and show the utility of spatiotemporal Eh dynamics as footprints reflecting functioning of benthic detrital food webs.

Key words: decomposition, aquatic invertebrates, bioturbation, functional traits, bacterial community structure, redox potential.
community structure remain ill-defined, especially in freshwater sediments.

Detritivorous invertebrates display a wide variety of bioturbation/feeding traits that differentially affect sediment biogeochemistry, bacterial activity, and detritus processing (Mermillod-Blondin et al. 2002a, Jonsson and Malmqvist 2003, Nogaro et al. 2009). Invertebrates can be categorized by the way they affect the physicochemical and biogeochemical properties of the sediment (François et al. 1997b, Gérino et al. 2003, Nogaro et al. 2009). Invertebrates may act as: 1) biodiffusors whose surface activity results in random downward mixing; 2) upward conveyors and 3) downward conveyors, whose activities move sediment vertically upward or downward, respectively; 4) regenerators that create open burrows that fill with surface particles when abandoned; and 5) gallery diffusors that create galleries of actively irrigated burrows. Identifying trait-specific signatures of sediment reworking would greatly enhance our capabilities to explain and predict ecosystem responses to changes in environmental pressure and declines in diversity, but identifying these interactions is methodologically challenging and laborious (Solan et al. 2004, Naeem and Bunker 2009, Birchenough et al. 2012). Spatial and temporal redox potential (Eh) profiles have been used to reflect biogeochemical processes and functioning of sediments in response to bioturbation (Hunting and van der Geest 2011, Vorenhout et al. 2011) and might provide useful biogeochemical signatures of species-specific bioturbation/feeding activities.

Our objectives were to evaluate whether bioturbation/feeding traits of aquatic invertebrate species differentially affect detritus processing and benthic microbial community structure and to test the utility of Eh profiles as biogeochemical signatures of types of bioturbation in laboratory microcosms.

Methods

Microcosms and test organisms

Microcosms.—We tested the effects of bioturbation/feeding activity on bacterial community structure, detritus processing, and redox geochemistry in the immediate environment of the invertebrates in laboratory microcosms. We constructed microcosms from sterilized 50-mL glass vials (25-mm diameter) filled with fine-grained, ignited quartz sand as mineral substrate (12.5 g, grain size: 0.1–0.5 mm, total sediment depth: 18 mm). We assumed that the size of the microcosms did not affect invertebrate behavior. We used 8 mg of freeze-dried, ground, and sieved stinging nettle (Urtica dioica L., <500 µm particle size) as detritus. This plant often dominates the riparian zone of aquatic systems (Stief 2007). We filled microcosms with 35 mL of Dutch Standard Water (DSW; a standardized synthetic analog of common Dutch surface waters). DSW contains 200 mg CaCl$_2$·2H$_2$O, 180 mg MgSO$_4$·7H$_2$O, 100 mg NaHCO$_3$, and 20 mg KHC$_2$O$_3$·H$_2$O demineralized H$_2$O (pH 8.1, hardness 210 mg/L CaCO$_3$, alkalinity 1.2 meq/L). We gently aerated the overlying water through needles without disturbing the sediment.

Treatments and controls.—We compared effects of 5 invertebrate species in microcosms with and without bacterial inocula on organic matter processing, bacterial community structure and activity, and sediment Eh profiles. Each treatment and control was replicated 7 times (140 microcosms). We inoculated half of the microcosms with a bacterial consortium (see below). We added individuals of a single invertebrate species (see below) to half of the microcosms (7 microcosms/species) with a bacterial consortium (invertebrate treatment) and to half of the sterile microcosms (control for contribution of invertebrate-derived bacteria). Microcosms inoculated with a bacterial consortium and no invertebrates were negative controls for the invertebrate treatments. We used sterile microcosms without invertebrates to calculate organic matter content at the initial time point in calculations of decomposition rate.

Bacteria.—We used bacterial communities from metabolically and taxonomically distinct strains of bacteria isolated from aquatic sediments. We assembled communities from overnight cultures of Azospirillum brasilense, Bacillus subtilis, Paenibacillus polymyxa, Pseudomonas putida, Sphingomonas paucimobilis, Micrococcus luteus, Streptomyces antibioticus, Pseudomonas stutzeri, Flavobacterium sp., Aeromonas salmonicida, Paracoccus pantotrophus, and Aminobacter aminovorans (obtained from the Fungal Biodiversity Centre, CBS-KNAW, Utrecht, The Netherlands), all grown in brain–heart broth (Merck, Darmstadt, Germany) and peptonized milk nutrient (Sigma–Aldrich, St. Louis, Missouri) (ratio 100:15). We standardized bacterial biomass for each strain by dilution to obtain an optical density at 600 nm (OD600) of 0.2. We inoculated each microcosm (except for the sterile microcosms) with 1 mL of bacterial suspension.

Invertebrates.—We selected aquatic invertebrates based on consensus regarding their bioturbation traits in the primary literature, availability, and performance under culture and laboratory conditions. We used 5 invertebrate species that represented 3 types of bioturbators as outlined by Usseglio-Polatera et al. (2000) and 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 non-
biting 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 procedure**

At the start of the experiment, we inoculated
microcosms with bacterial communities and left them
undisturbed to allow stratification of the sediment,
succession of the bacterial community, and partial
degradation of detritus. We added invertebrates to the
appropriate microcosms (*n* = 7 microcosms per species)
40 h after bacterial inoculation. To standardize inver-
tebrate biomass, we used relationships between length
(for *C. riparius*, *A. aquaticus*, and *G. pulex*) or fresh mass
(*Tubifex* spp. and *L. variegatus*) and dry mass (DM)
based on 12 to 30 individuals/species. We added equal
initial DM of invertebrates to the microcosms (mean ±
SD, 0.35 ± 0.03 mg DM/microcosm). We used 1 small
individual of *A. aquaticus* and 6 *G. pulex* (5–7 mm in
length) per microcosm, and 3 or 4 individuals of *C.
riparius*, *Tubifex* spp., and *L. variegatus/microcosm*. After
5 d, we evaluated the influence of the invertebrate
species on detritus processing, bacterial activity, bacte-
rial community structure, and development of Eh.

**Organic matter processing**

We characterized detritus processing as sediment
mass loss on ignition (LOI) and increase in DOC in the
overlying water at the end of the experiment. After we
collected 2 mL of sediment from each microcosm for
measurement of bacterial activity and community
structure (see below), we oven dried the remaining
sediment and combusted it at 550°C for 24 h. We
calculated LOI as mass loss relative to mass of sediments
in sterile controls that did not contain invertebrates. We
measured DOC as absorbance at 280 nm in the overlying
water. We tested the data for normality (Lilliefors) and
used 1-way analysis of variance (ANOVA) and Tukey’s
Honestly Significant Difference (HSD) post hoc test
(Matlab version 7.2, Mathworks, Boston, Massachusetts)
to identify differences among invertebrate treatments in
inoculated microcosms, including inoculated controls
with no invertebrates.

**Bacterial activity and community structure**

We assessed bacterial activity in the sediment by
measuring electron transport system activity (ETSA)
following reduction of 2-(p-iodophenyl)-3-(p-nitro-
phenyl)-5-phenyl tetrazolium chloride (INT) to form-
azan (INTF) sensu Smith and McFeters (1997). In
brief, we collected 1 mL of sediment, vortexed it with
1 mL of overlying medium, and centrifuged (short
spin) it to deposit coarse material. We assayed the
supernatants (400 μL porewater with suspended
bacteria) for ETSA with procedures recently described
by Hunting et al. (2010). We used the same statistical
analysis described above.

We collected sediment for deoxyribonucleic acid
(DNA) analysis with 1 mL pipettes. We centrifuged
the samples, air-dried them, and stored them at
−20°C until analysis. We extracted bacterial DNA
from 2 g of sediment using PowerSoil extraction kits
(Mo-Bio, Carlsbad, New Mexico). We assayed bacte-
rial community structure in 3 replicates of each
inoculated and sterile invertebrate treatment and 2
inoculated microcosms with no invertebrates with
ribosomal intergenic spacer analysis (RISA) with
universal 16–23S bacterial primers and polymerase
chain reaction (PCR) amplification as described by
Danovaro et al. (2006). We separated PCR-amplified
fragments on 3.5% polyacrylamide gels and stained
the gels with ethidium bromide. We analyzed RISA
banding patterns with a Jaccard-based cluster analysis
and 1-way analysis of similarity (ANOSIM), followed
by a permutation based, Bonferroni-corrected pair-
wise comparison using PAST (Hammer et al. 2001).

**Redox potential profiles**

We visualized effects of invertebrate species on
sediment geochemistry as vertical profiles of Eh
recorded over time. We measured Eh in 3 replicates
of each invertebrate treatment in inoculated micro-

We constructed Eh microelectrodes from Au-
plated printed circuit board and placed them perma-
nently in the middle of the sediment cores to allow
high-resolution measurement of Eh in subsurface
sediments (each mm [0–7-mm] depth, 2-mm width,
every 15 min) throughout the experiment. During the
preincubation (first 40 h), 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 and generated
contour charts with linear interpolation (DeltaGraph,
version 5.0; Red Rock Software, Salt Lake City, Utah). We used a general linear model (GLM) approach to analysis of covariance (ANCOVA) to compare measurements of Eh among invertebrate treatments (Engqvist 2005). The dependent variable was mean Eh in time at each depth, and depth was the covariate. In the ANCOVA, invertebrate treatments did not differ in slopes \( (p = 0.14) \), a result that could have indicated depth dependence. Therefore, we removed the interaction term to test for the effect of invertebrate treatment with a Tukey’s HSD post hoc test.

Results

Bacterial activity and detritus processing did not differ between inoculated and sterile microcosms containing invertebrates for any invertebrate species (unpaired \( t \)-tests, \( p > 0.35 \); Fig. 1A–C), a result suggesting that invertebrates contributed strongly to bacterial activity and detritus processing. Bacterial activity and detritus processing were higher in all inoculated microcosms with invertebrates than in inoculated microcosms without invertebrates (Fig. 1A–C). However, the magnitude of enhancement depended on the invertebrate species. *Chironomus riparius*, *L. variegatus*, and *Tubifex* spp. increased LOI (Fig. 1A) and DOC (Fig. 1B) by 60 to 400% and increased bacterial activity by 30 to 600% relative to inoculated controls without invertebrates. *Asellus aquaticus* and *G. pulex* processed, on average, 60% more detritus than the other invertebrate species (Fig. 1A, B) and sustained 50 to 500% higher bacterial activity than the other invertebrate species (Fig. 1C).

RISA banding patterns were variable, and the bands derived from inocula were overlaid by bands derived from invertebrate-associated bacteria. That is, bands appeared in microcosms with invertebrates that were not visible in microcosms containing only inocula. Approximately 50 to 60% of the bacterial community in microcosms with *Tubifex* spp., *L. variegatus*, and *C. riparius* and 30 to 35% of the bacterial community in microcosms with *A. aquaticus* and *G. pulex* originated from the invertebrates. In the cluster analysis (Fig. 2), bacterial communities in sterile microcosms with invertebrates were not necessarily separated from bacterial communities in inoculated microcosms with the same invertebrate species (ANOSIM, \( p > 0.4 \); Fig. 2). Bacterial commu-

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Mean (±1 SD) loss of particulate organic matter on ignition (LOI) (A), dissolved organic C (DOC) in the overlying water column (absorbance \( [A] \) at 280 nm) (B), and bacterial electron transfer system activity (ETSA) (C) in *Gammarus pulex, Asellus aquaticus, Tubifex* spp., *Lumbriculus variegatus*, and *Chironomus riparius* treatments in microcosms with bacterial inocula, without bacterial inocula, and in controls (inoculated microcosms without invertebrates). Bars with the same letters are not significantly different (1-way analysis of variance, Tukey’s Honestly Significant Difference post hoc test, \( n = 7, p < 0.05 \)).
Development of sediment Eh profiles over time differed between inoculated microcosms with and without invertebrates (see Fig. 3 for 1 representative replicate of each treatment). Replicates were very similar within all invertebrate treatments. Averaged Eh at each depth differed among invertebrate treatments (ANCOVA, $F = 60.05$, $p < 0.05$). Eh profiles differed between microcosms with sediment-dwelling biodiffusors ($A. aquaticus$ and $G. pulex$) and microcosms with burrowing invertebrates ($Tubifex$ spp., $L. variegatus$ and $C. riparius$) (Tukey HSD test; Fig. 4). Eh was higher at the sediment–water interface in microcosms with $G. pulex$ and $A. aquaticus$ than in microcosms with $Tubifex$ spp., $L. variegatus$, and $C. riparius$ (Fig. 4).

**Discussion**

Invertebrates strongly contributed to bacterial activity and detritus processing and overruled effects of the starting bacterial inocula. Enhancement of bacterial activity and detritus processing by invertebrates has been observed in a number of studies (van de Bund et al. 1994, Wieltschnig et al. 2008, Hunting and van der Geest 2011). Detritivorous invertebrates incorporate large amounts of bacterial biomass, but this loss is often (over-) compensated by the stimulatory effects of nutrient excretion, partial degradation of organic matter, and irrigation in the presence of invertebrates (Traunspurger et al. 1997, Meysman et al. 2006). Similar mechanisms probably were responsible for the stimulatory effects observed in our study.

The magnitude of bacterial activity and detritus processing depended on invertebrate bioturbation/feeding trait, and the presence of sediment-dwelling biodiffusors resulted in higher bacterial activity and detritus processing than did the presence of burrowing organisms. This result differs from the outcomes of other studies (Mermillod-Blondin and Rosenberg 2006, Meysman et al. 2006) in which burrowing organisms were identified as the main bioturbators affecting organic matter processing in sediments. This discrepancy may be a consequence of differences in invertebrate densities among studies. Other investi-
Fig. 3. Redox potential (Eh) profiles in depth (7 mm) and time of incubation in microcosms with *Gammarus pulex*, *Asellus aquaticus*, *Chironomus riparius*, *Tubifex* spp., or *Lumbriculus variegatus* compared to in inoculated microcosms without invertebrates (−). The contour plots are representative of 3 replicates for each treatment.
gators used natural densities of invertebrates. Oligochaetes and *C. riparius* often are far more abundant (100:1) than *A. aquaticus* and *G. pulex* in natural systems, resulting in a 25× difference in relative abundances of burrowing and sediment-dwelling invertebrates between our model system and those in other studies. Thus, density appears to influence invertebrate effects on sediment processes. This result highlights the need for standardization by invertebrate mass or biovolume (sensu Michaud et al. 2005) when characterizing invertebrate communities. In addition, we focused on the top layer (25 mm) of the sediment. The burrowing organisms used in our study often act in deeper layers of natural sediments, and these effects were not captured in our microcosms. Thus, the relative contribution of burrowing organisms to decomposition may have been underestimated in our microcosms.

Bacterial community structure was strongly affected by invertebrate bioturbation/feeding traits, i.e., bacterial communities differed between microcosms with sediment-dwelling invertebrates and those with burrowing species. Part of the bacterial community (up to 50%) originated from the invertebrates, a result suggesting that bacteria introduced by invertebrates strongly affected bacterial community structure and functioning. In addition, similarity in microbial community structure mirrored similarity in sediment Eh conditions. Eh measurements reflect an ensemble of system-specific redox reactions, so how Eh values obtained in our study are related to conditions in natural sediments is not known. Nonetheless, our results suggest that invertebrate bioturbation/feeding activities shape redox conditions and microbial community structure.

Only a few investigators have addressed the effect of habitat heterogeneity and the presence of macroinvertebrates on microbial diversity in sediments. Burrow walls might harbor unique microbial consortia because physicochemical conditions in burrows are generally more stable than those at the frequently disturbed sediment surface (Kristensen and Kostka 2005). Papaspyrou et al. (2005, 2006) reported results that support this hypothesis, but evidence is increasing that similarity in bacterial community structure and metabolic activity coincide with similarity in geochemical conditions, in particular redox conditions (Bertics and Ziebis 2009, Hunting and van der Geest 2011), as observed in our study.

Our results suggest tight coupling between invertebrate bioturbation/feeding activities and redox conditions, microbial communities, and detritus processing. Hence, our findings support the proposal that aquatic invertebrate functional traits are linked to ecosystem processes (Hillebrand and Matthiessen 2009, Nogaro et al. 2009). A key issue in trait-based approaches to research on biodiversity–ecosystem function is identifying and quantifying those functional traits relevant to ecosystem properties (Naem and Bunker 2009). We were able to use Eh profiles to

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**Fig. 4.** Mean (*n* = 3) redox potential (Eh) at each depth in microcosms with *Asellus aquaticus, Gammarus pulex, Chironomus riparius, Tubifex spp.*, or *Lumbriculus variegatus* compared to inoculated microcosms without invertebrates (control). Depth dependence was defined by the slopes and did not differ significantly among treatments (general linear model–analysis of covariance (GLM–ANCOVA, *p* = 0.14). Therefore, we excluded Eh × depth interactions and detected invertebrate treatment effects with a Tukey’s Honestly Significant Difference post hoc test (*p* < 0.05). Lines with the same letter are not significantly different.
provide quantitative signatures that demonstrated the effect of invertebrate activity on biogeochemical processes and detritus processing. We showed that invertebrates with different functional traits had very different effects on spatial and temporal properties of sediment Eh. More important, invertebrates with similar bioturbation/feeding traits produced comparable Eh profiles in space and over time. Eh profiles produced by sediment dwellers (A. aquaticus and G. pulex [biodiffusers]) and burrowing organisms (Tubifex spp., L. variegatus [upward conveyors] and C. riparius [gallery diffusors]) differed strongly. Moreover, Eh profiles produced by upward conveyors and gallery diffusors also differed. Therefore, we propose using Eh profiles as readily obtained footprints of invertebrate bioturbation/feeding activities.

How ecosystem functional responses observed in our model system might change in multispecies invertebrate assemblages or under natural conditions is not clear. However, the differing effects of invertebrates with different bioturbation/feeding activities on bacterial community structure and detritus processing suggest that these traits could be used to study effects of faunal diversity on ecosystem processes in fresh water, as well as in marine (Covich et al. 2004, Solan et al. 2004, Birchenough et al. 2012) and grassland ecosystems (Klump and Soussana 2009, Mulder and Elser 2009). We showed that aquatic invertebrate bioturbation/feeding traits mediate detritus processing, redox geochemical characteristics of the sediment, and microbial community structure. Our results support the trait-based framework linking bioturbation to ecosystem properties and processes in a wide range of habitats (Mermillod-Blondin 2011).

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