Vol. 36: 271–284, 2004 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published September 14

Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment

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ABSTRACT: Benthic invertebrates play a key role in the physical, chemical, and biological properties of the marine water-sediment interface. The influences of invertebrates on biogeochemical processes have mainly been attributed to their sediment reworking and bioirrigation activities. The aim of this study was to compare the influences of bioturbation activities by 3 dominant species of shallow water habitats (*Cerastoderma edule*, *Corophium volutator*, and *Nereis diversicolor*) on microbial communities and biogeochemical processes in sediment cores. *C. edule* acted as a biodiffuser, mixing surface particles in the top 2 cm of the sediment. Despite this mixing activity, this species had little effect on O_2 consumption, water exchange between the water column and the sediment, microbial characteristics, and release of nutrients from the sediment. In contrast, *C. volutator* and *N. diversicolor* produced burrows in the sediment that allowed transport of surface particles into biogenic structures. These 2 species doubled the solute exchange between the water column and the sediment. Such modifications of sediment structure and solute transport increased the O_2 consumption and the release of nutrients from the sediment. Both *C. volutator* and *N. diversicolor* stimulated the microbial communities as indicated by higher percentages of active bacteria. Reduction of the numbers of sulphate reducing bacteria was observed when the 3 invertebrates were present and could be attributed to the penetration of O_2 due to animal activities. *N. diversicolor* had a greater influence than *C. volutator* on pore water chemistry, ammonium release, and active bacteria. As *N. diversicolor* burrowed deeper in the sediment than *C. volutator*, it irrigated a greater volume of sediment. The modes of sediment reworking and structure building, irrigation behaviour, and burrowing depths were factors sufficient to assign the 3 species into different functional groups.

KEY WORDS: *Nereis diversicolor* · *Corophium volutator* · *Cerastoderma edule* · Functional groups · Bioturbation · Microbial activity · Organic matter processing

INTRODUCTION

In aquatic ecosystems, benthic fauna play a key role in organic matter processing and nutrient cycling at the water–sediment interface (Rhoads 1974, Aller 1983, Hansen & Kristensen 1998, Rosenberg 2001). Several studies have shown the ecological importance of infaunal sediment reworking, irrigation, burrow, and tube structures for biogeochemical processes in lacustrine (Krantzberg 1985, Stief & de Beer 2002), marine (Reichardt 1988, Kristensen et al. 1991, Gilbert et al. 1998, 2003), and river sediments (Mermillod-

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Blondin et al. 2003). In soft marine bottom habitats, the influence of a benthic species on organic matter mineralization usually depends on its mode of bioturbation (sediment reworking and bio-irrigation behaviour). Pelegri & Blackburn (1995) demonstrated that 3 species exhibiting different bio-irrigation activities influenced differently the N-cycle in marine sediment. Similarly, the deep bioirrigation of *Arenicola marina* produced a greater carbon mineralization than the shallower irrigation activity of *Nereis diversicolor* (Banta et al. 1999). Christensen et al. (2000) also demonstrated that a suspension-feeding polychaete (*Nereis diversicolor*) and a non-suspension feeding polychaete (*Nereis virens*) had very different influences on sediment biogeochemistry due to their different feeding behaviours.

To quantify bioturbation activity, sediment particle tracers were commonly used to mark particle migration due to invertebrate activities (Aller & Cochran 1976, Mahaut & Graf 1987, Gerino et al. 1998). Tracer experiments can be easily coupled with bioturbation models to determine the specific influence of each invertebrate on sediment mixing (François et al. 1997, 2002). This modelling approach allowed the classification of marine invertebrates into different functional groups (see review in Gerino et al. 2003). However, although invertebrates can be grouped in several functional types, no study has tried to determine how the mode of sediment mixing reflects the influence of invertebrates on biogeochemical processes.

Invertebrate bioturbation potentially affects bacterial communities by modifying the supply of nutrients and O_2 to micro-organisms (Yingst & Rhoads 1980). Therefore, quantification of specific populations of micro-organisms in the sediment can be a very efficient way to analyse the influence of bioturbation on physico-chemical conditions and biogeochemical processes in the sediment. In this way, molecular analysis using labelled rRNA-targeted nucleic acid probes allows an in situ identification of bacteria in their natural habitat (Amann et al. 1997). This molecular technique is commonly used to analyse the diversity of bacterial communities in soils (Zarda et al. 1997, Christensen et al. 1999), streams (Araya et al. 2003), and marine sediments (Boetius et al. 2000). However, to our knowledge, molecular probes have never been used to analyse the role of the bioturbation process on bacterial communities.

Thus, the main objective of the present study is to analyse the bioturbation process (sediment reworking and the bioirrigation process) induced by 3 species (*Nereis diversicolor* [Polychaeta], *Corophium volutator* [Amphipoda] and *Cerastoderma edule* [Bivalvia]) and its influence on biogeochemical processes (oxygen and solute fluxes), pore water chemistry (ammonium, nitrate, phosphate, and silicate), and bacterial communities (total number of bacteria, active eubacteria, and active sulphate-reducing bacteria).

MATERIALS AND METHODS

Invertebrates studied. The 3 selected species are among the dominant species in the Gullmarsfjord (Sweden) and presented different sediment reworking and bio-irrigation activities. *Cerastoderma edule* is usually found at 2 to 7 cm depth in the sediment. The animal is a suspension feeder that pumps water from the overlying water and back through its 2 siphons at the water-sediment interface. It commonly occurs in densities of 15 to 300 individuals m^{-2} (Muus 1967, Rasmussen 1973) but higher densities of juveniles (6000 to 14 000 individuals m^{-2}) have been reported in the Skagerrak (Möller & Rosenberg 1983). *Corophium volutator* lives in irrigated U-shaped burrows at 2 to 4 cm deep. The burrow acts as a specific habitat for micro-organisms that is influenced by redox oscillations due to animal irrigation (Aller 1994). The mean abundance of *C. volutator* varies from 3000 to 45 000 individuals m^{-2} in the Skagerrak (Möller & Rosenberg 1982). *Nereis diversicolor* lives in mucus-lined gallery of burrows extending 6 to 12 cm into the sediment (Davey 1994). *N. diversicolor* can act both as deposit-feeder and as filter-feeder (Riisgard 1991). It produces, by muscular movements of the body, a more or less continuous current of water carrying oxygen and food particles into the burrow (Riisgard 1991, Pelegri & Blackburn 1995). *N. diversicolor* is common in shallow waters and has been found in natural numbers of 500 to 5000 individuals m^{-2} (Möller 1985, Vedel & Riisgard 1993).

Study site. Sediment was collected in June 2002 (see timetable in Table 1) in a shallow bay located at the mouth of the Gullmarsfjord (58° 15′ N, 11° 28′ E) on the Swedish west coast. The bay had a silt-sand sediment (60% of fine sand and 40% of silt) mixed with shell debris, with an organic content varying spatially between 0.8 and 1.7% (Pihl 1986). To reduce the natural heterogeneity and to obtain equal starting conditions, the sediment was homogenized before use. Sample from the upper 8 to 10 cm of the sediment was sieved through a 1 mm mesh to remove macrofauna and larger particles (shells). Intact specimens of *Cerastoderma edule* (30 mm long), *Corophium volutator* (5 to 8 mm long), and *Nereis diversicolor* (60 to 100 mm long) were collected in the Gullmarsfjord. For acclimation, these invertebrates were kept under similar experimental conditions (particle grain sizes and food) for more than 7 d before introduction into the cores.

Sediment cores. Sediment microcosms were established by transferring the homogenized sediment into plexiglass cores (28 cm long and 10 cm internal diameter) to a depth of ~18 cm. Twelve cores were placed in a dark room at 14°C. A continuous water (salinity: 32 psu, controlled temperature: 14°C) flow-through system (120 ml min^{-1}) was installed. The turnover rate of the overlying water (10 cm deep) was between 6 and 7 min. Macroinvertebrates were introduced to the microcosms 10 d after the sediment installation when recovery from the sediment disturbance was complete.

Experimental setup. Four treatments were performed with 3 replicate cores per treatment: (1) without macrofauna (control), (2) 2 *Cerastoderma edule* per core, (3) 40 *Corophium volutator* per core, and (4) 5 *Nereis diversicolor* per core. The macrofaunal densities tested were equivalent to 250, 5100, and 640 individuals m^{-2} for *C. edule*, *C. volutator*, and *N. diversicolor*, respectively. These densities were close to the average densities observed in the Skagerrak (see above). Chemical measurements in the overlying water, on Days –2 and –1 before the faunal introduction, confirmed that O_2 uptake (950 ± 51 µmol h^{-1} m^{-2}, mean ± SD), ammonium (16.82 ± 3.49 µmol h^{-1} m^{-2}), nitrate (8.42 ± 1.37 µmol h^{-1} m^{-2}), phosphate (0.30 ± 0.11 µmol h^{-1} m^{-2}), and silicate (4.68 ± 1.02 µmol h^{-1} m^{-2}) released from the sediment were comparable in the 12 cores. Most animals burrowed rapidly (<2 h) into the sediment, although some individuals of *C. edule* moved at the sediment surface and took more time (from 1 to 3 d) to burrow into the sediment matrix.

During the 20 d of the experiment, physical, chemical and bacteriological parameters were measured in the cores to quantify the effect of macrofauna (Table 1). Exchanges of DIN (dissolved inorganic nitrogen; NO_2^- + NO_3^- and NH_4^+), phosphate (PO_4^{3-}), and silicate (SiO_4^{4-}) were measured on Day 8 of the experiment. Water exchange was measured on Day 10 of the experiment using bromide as the tracer of water. O_2 consumption within cores was estimated after 12 d of the experiment. Bacteria attached to the sediment were analyzed on samples randomly taken with sleeved plexi-core (30 mm in diameter) in each experimental unit on Day 15. Surface particle redistribution, pore water chemistry (NO_2^- + NO_3^-, NH_4^+, PO_4^{3-}, and SiO_4^{4-}), and the nitrogen and organic carbon content of the sediment were sampled at the end of the experiment (Day 20).

Table 1. Time table of the experimental procedure

Preparations and measurements	Date	Day code
Collection of the sediment	20 June	Day –13
Core preparation	23 June	Day –10
Animal collection	26 June	Day –7
Measures of the variability among cores before animal introduction:		
for O_2 fluxes	1 July	Day –2
for solute fluxes	2 July	Day –1
Introduction of the animals in cores	3 July	Day 0
Measures of solute fluxes	11 July	Day 8
Measures of water transport	13 July	Day 10
Measures of O_2 fluxes	15 July	Day 12
Collection of sediment for microbial analyses	18 July	Day 15
Core opening and collection of sediment for luminophores, pore water content and pore water chemistry	23 July	Day 20

Physical, chemical, and bacterial measurements. ***Sediment reworking:*** Sediment reworking in the cores was quantified by the luminophore tracer technique (Mahaut & Graf 1987, Gerino 1990). On Day 0 of the experiment, 1 g of 100 to 150 µm luminophores (natural sediment particles dyed with fluorescent paint) were deposited on the sediment surface after the invertebrate introduction. At the end of the experiment (20 d), water was removed, cores were opened, and sediment and luminophores were sampled. The top 9 cm of each core were sampled in layers of 0.5 cm. The total sediment collected at each layer was homogenised and a 1 g sub-sample was dried at 50°C for luminophore counting. The numbers of luminophores were estimated under a UV light microscope and converted into percentage of tracer in each sediment slice. The impact of invertebrates on particulate matter mixing was quantified using the biodiffusion and the gallery-diffuser models developed by François et al. (1997, 2002).

The biodiffusion model uses ordinary differential equations to simulate the diffusive sediment transport due to animals which move sediment particles in a random manner over short distances. It is characterized by a biodiffusion rate expressed in d^{-1}. The parameters of the model are defined in Table 2 and the functional diagram and the mathematical equations are presented in Table 3.

The gallery-diffuser model is used to simulate the sediment mixing due to species whose main activities are to dig systems of galleries, tubes, or burrows in sediment and to practice bioirrigation. This activity leads to the combination of a biodiffusive mixing of sediment in the surface layers and a non local transport of matter from the surface to the deep part of the biogenic structures. The model uses 2 parameters, a biodiffusion rate (ex-

Table 2. Parameters of the biodiffusion and gallery-diffuser models

Variable	
$Q(r, t)$	Quantity of tracer contained in the cell r at time t (mass)
Biological parameters	
Position parameters	
n	Depth of the organism mixing zone (no. of cells)
m_b	Height of the diffusion zone of the organism (no. of cells)
Mixing parameters	
R_{D_b}	Biodiffusion rate of the organism (time^{-1})
R_{NL}	Non local transport rate of the organism (time^{-1})

pressed in d^{-1}) and a non-local transport rate (expressed in d^{-1}). The parameters of the model are defined in Table 2 and the functional diagram and the mathematical equations are presented in Table 4.

We used the following transformation to make the biodiffusion rate independent of the cell size and to express it as it appears in the literature:

$$D_b = R_{D_b} \times \delta^2$$

where D_b is the biodiffusion coefficient (cm d^{-1}), R_{D_b} is the biodiffusion rate as used in our models (d^{-1}) and δ is the thickness of the sediment layers (cm) (François et al. 2002). The non-local transport was expressed in percentage of sediment removed from the surface and buried at depth per day (% d^{-1}).

The transport coefficients are determined by comparing the experimental and simulated luminophores profiles using the least-squares criterion (by minimizing the sum of the squared differences between observed and calculated percentages of tracer at each depth).

Benthic oxygen flux: To measure the O_2 flux, the water flow system was stopped for all cores. Cores were sealed with gas-tight plastic lids during flux measurements. The water column in each core was mixed by magnetic stirring (60 rpm). Hourly-based flux rates

Table 3. Functional diagram and mathematical equations of the biodiffusion model. For the functional diagram we represent a discretized sediment core inhabited by a biodiffuser that reworks particle down to the nth sediment layer. Arrows show fluxes of sediment between layers

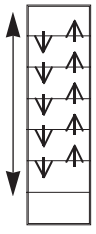
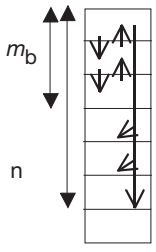
Sediment surface	Biodiffusion model	
	Row 1	$\frac{dQ(r,t)}{dt} = -R_{D_b}Q(r,t) + R_{D_b}Q(r+1,t)$
	Row 2 to (n–1)	$\frac{dQ(r,t)}{dt} = -2R_{D_b}Q(r,t) + R_{D_b}Q(r-1,t) + R_{D_b}Q(r+1,t)$
	Row n	$\frac{dQ(r,t)}{dt} = -R_{D_b}Q(r,t) + R_{D_b}Q(r-1,t)$

Table 4. Functional diagram and mathematical equations of the gallery-diffuser model. For the functional diagram we represent a discretized sediment core inhabited by a gallery-diffuser that reworks particle down to the nth sediment layer. There is biodiffusive mixing in the m_b first layers and non local transport from the first to the (m_b + 1) to n layers. Arrows show fluxes of sediment between layers

Sediment surface	Gallery-diffuser model	
	Row 1	$\frac{dQ(r,t)}{dt} = -R_{D_b}Q(r,t) + R_{D_b}Q(r+1,t) - R_{NL}Q(r,t)$
	Row 2 to (m_b – 1)	$\frac{dQ(r,t)}{dt} = -2R_{D_b}Q(r,t) + R_{D_b}Q(r-1,t) + R_{D_b}Q(r+1,t)$
	Row m_b	$\frac{dQ(r,t)}{dt} = -R_{D_b}Q(r,t) + R_{D_b}Q(r-1,t)$
	Row (m_b + 1) to (n)	$\frac{dQ(r,t)}{dt} = -R_{NL}\frac{Q(1,t)}{(n-m_b)}$

were determined from changes over time in the concentration of O_2 in the water column (Kristensen & Hansen 1999). Flux rates were obtained from linear regressions of solute concentrations versus time as exemplified in Fig. 1 for ammonium fluxes. Tests showed that decrease of O_2 concentrations from the start to the end of the incubation did not lead to concentration below 80% of the initial concentrations. O_2 concentrations of the samples were analyzed within 1 h by the Winkler titration technique.

Nutrient exchange fluxes: The water flow system was stopped for all cores and 100 ml water above the sediment was removed to avoid a loss of water from the water column during stirring. In the remaining water, stirring (60 rpm) was maintained. The cores were then incubated for 8 h and water samples (35 ml) were taken every 2 h and immediately frozen (–20°C) for later analysis. The exchange rates of NH_4^+, NO_3^- (NO_3^- + NO_2^-), phosphate and silicate across the sediment-water interface were calculated from changes over time in the concentration of each species in the water column.

Water movement from the water column to the sediment: Bromide ([Br^-] = 16 ± 0.35 mM) was added to the water column. During an incubation period of 4 h with the stirring system, samples (5 ml) were taken at 0, 1, 2, 3, and 4 h. The samples were immediately filtered and frozen. Br^- was analyzed by ion exchange chromatography (Dionex, Ion Pac AS4A) with a 1.7 mM $NaHCO_3$, 1.8 mM Na_2CO_3 eluent, a flow rate of 2 ml min^{-1}, and a 0.07% solution of concentrated H_2SO_4 regenerant at a flow rate of 3.5 ml min^{-1}. Peak areas of samples were compared to standards. The movement of water from the water column to the sediment was estimated as the changes over time in the concentration of Br^- in the water column (Forster et al. 1999).

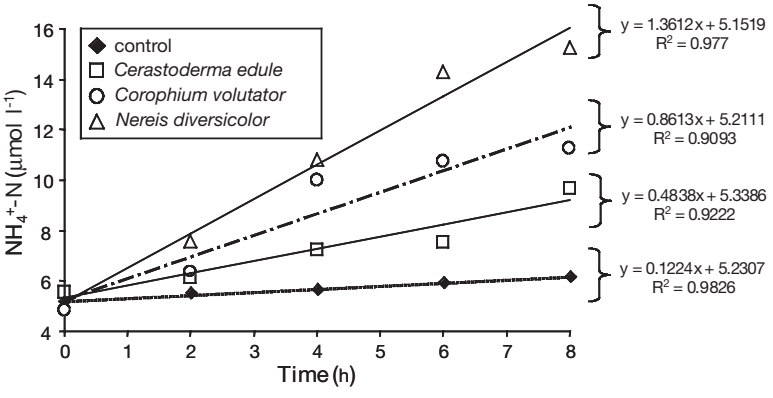


Fig. 1. Concentration of ammonium in relation to time during the incubation of 4 cores (each treatment being represented by 1 core). The slopes obtained from the regression lines were used as estimates of the flux rates of ammonium from the sediment to the water column

Pore water and sediment chemistry: During core opening, sub-samples were taken for the luminophore analyses and used for determination of porosity (weight loss after 24 h at 105°C) and analysis of the pore water chemistry. Sub-samples of sediment were collected in each core for 6 layers: 0–1, 1–2, 2–3, 3–4, 4–6, and 6–8 cm. Pore water was obtained by centrifugation (30 min at 5000 rpm, 4000 × *g*). The supernatant (5 ml) was collected and frozen (–20°C). NO_2^- + NO_3^-, NH_4^+, PO_4^{3-} and SiO_4^{4-} concentrations in pore were measured by standard methods with a TRAACS 800, 4 channel automated nutrient analyzer (Grasshoff et al. 1983). The remaining centrifuged sediment was dried at 60°C overnight and used to measure total organic carbon (TOC) and total nitrogen (TN) on a NA 1500 NC Carlo Erba elemental analyzer (Fisons) according to Hedges & Stern (1983).

Bacterial measurements: For each sleeved plexicore (30 mm in diameter) sampled on Day 15, 2 g of wet sediment were immediately collected from 5 layers: 0–0.5, 1–1.5, 2–2.5, 4–4.5, and 6.5–7.5 cm. Sediment samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 h. Fixed samples were subsequently washed twice in PBS. Afterwards, samples were stored in ethanol and PBS (50:50) at –20°C. Four ml of 0.1% pyrophosphate in PBS were added to 0.5 g of the sediment sample. All samples were then homogenized with a sonicator with a 2 mm-diameter probe (Sonicator XL 2020) at power 2.5 for 160 s at 50% of its active cycle. All suspensions were finally supplemented with the detergent NP-40 (Flucka) to a final concentration of 0.01%. Aliquots (10 µl) of fixed and dispersed samples spotted onto gelatine-coated slides were hybridized with Cy3-labeled oligonucleotide probes and concomitantly stained with the DNA intercalating dye DAPI (200 ng $µl^{-1}$, Sigma) according to Schönholzer et al. (2002). Probes were used to detect the Domains Bacteria (probe EUB 338, eubacteria) and bacteria belonging to the δ-subdivisions of Proteobacteria (SRB385, sulphate-reducing bacteria). Hybridizations were performed in 10 µl of hybridization buffer (0.9 M NaCl, 20 mM Tris/HCl, 5 mM EDTA, 0.01% SDS; pH 7.2) in the presence of 30% formamide, 1 µl of DAPI, and 1 µl of the probe (25 ng $µl^{-1}$) at 41°C for 2 h (Schönholzer et al. 2002). After hybridization, the slides were washed in buffer at 48°C for 15 min, rinsed with distilled water and air-dried. Slides were mounted with Citifluor solution and the preparations were examined at 1000× magnification with a DRMBE Leitz microscope fitted for epifluorescence with a high-pressure mercury bulb (100 W) and filter

sets A (for DAPI) and N2.1 (for Cy3). Bacteria from the samples were analyzed in 30 fields per sample with up to 50 cells per field. Numbers of DAPI- and Cy3-bacteria were counted separately from the same field in order to calculate the percentages of active (EUB/DAPI) and sulphate-reducing bacteria (SRB/DAPI) from each analyzed field.

Statistical analyses. For sediment reworking and flux rates, we tested treatment effect (treatments: control, *Cerastoderma edule*, *Corophium volutator*, and *Nereis diversicolor*) by a 1-way analysis of variance (ANOVA) using Statistica 5 TM (StatSoft). Regression analyses were used to determine the links between the water movement from the water column to the sediment (measured with bromide) and the biogeochemical fluxes (oxygen, ammonium, nitrate, phosphate, and silicate) in cores. For microbial parameters and pore water chemistry measured at different layers of the sediment, treatment effect was tested with a 1-way repeated measure ANOVA (RM ANOVA) with treatment as the main effect and depth as a repeated factor (within subject) because a measure at one depth was not independent from measures at adjacent depths. If significance was detected among treatments, Scheffé post hoc tests were performed to determine which treatment differed. When homoscedasticity was not observed, data were ln or square-root transformed to homogenise variances, whereas variables expressed as percentages (microbial variables) were arcsine transformed (Sokal & Rohlf 1995).

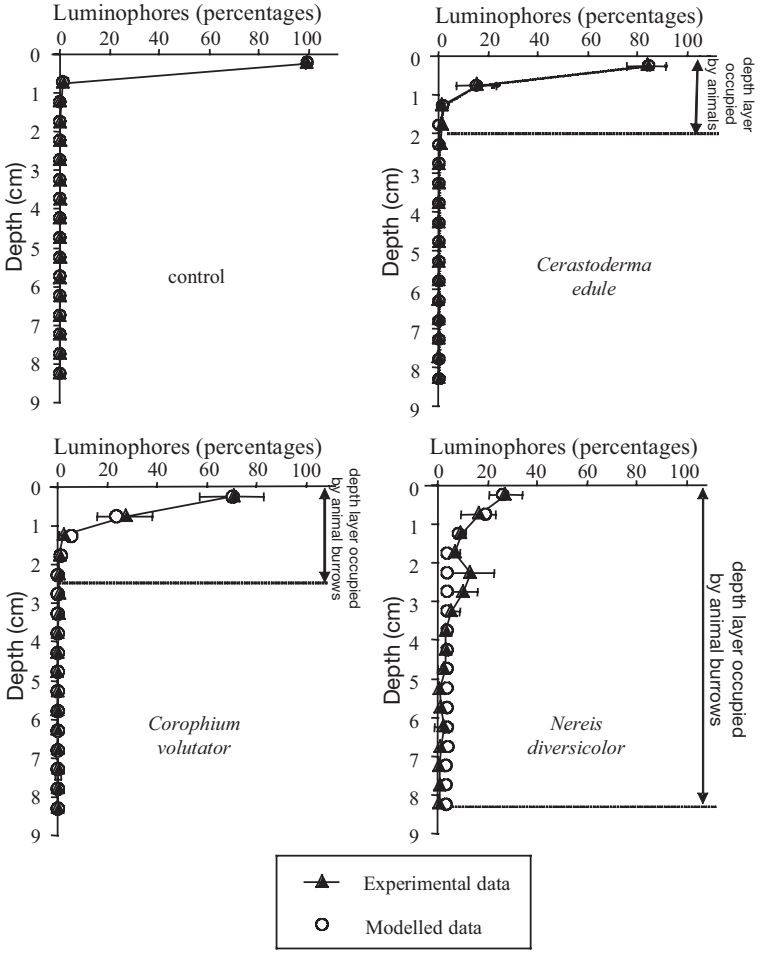


Fig. 2. Depth profiles of luminophores for the 4 treatments (means ± SD, n = 3 cores per treatment). Model profiles and the burrowing depth of animals (estimated from visual observations) were indicated for each treatment

RESULTS

Visual observations

In the control cores, a brownish coloured zone of 12 to 16 mm depth was observed. Below this oxidised zone, sediment was grey to greyish-black. In the bioturbated cores, biogenic structures produced by *Corophium volutator* and *Nereis diversicolor* extended the oxidized zone into the reduced sediment in the form of 2 to 5 mm thick oxidized wall linings around the burrows. During the sectioning of cores, burrows were observed down to 2–3 and 8–9 cm depth with *C. volutator* and *N. diversicolor*, respectively. The presence of *Cerastoderma edule* extended the oxidised zone in the areas where the bivalves burrowed. The observed depths of animal burrowing are indicated in Fig. 2 in association with data corresponding to the sediment reworking process.

Sediment reworking

Tracer distributions in control cores, cores with *Cerastoderma edule* and cores with *Corophium volutator* exhibited an exponential decrease with sediment depth typical of diffusion-like transports without any occurrence of non-local processes. In contrast, cores with *Nereis diversicolor* showed a peak of tracer between 2 and 3 cm in depth that revealed a non-local transport (Fig. 2). The percentage of tracer buried below 0.5 cm was less than 3% in the control while it was more than 10, 18 and 65% in sediment with *C. edule*, *C. volutator* and *N. diversicolor*, respectively. The maximum depths of occurrence of luminophores were 2, 3.5, and 8.5 cm for these 3 species, respectively.

The transport coefficients estimated with a

Table 5. Mixing rates of sediment estimated in the 4 treatments. Non-local and diffusive transport coefficients are indicated with average values ± standard deviations (n = 3 for each treatment)

Treatments	Non-local transport (% of tracer d^{-1})	Diffusion ($\times 10^{-3}$ cm^{-2} d^{-1})
Control	0 (0)	0.13 (0.14)
Cerasoderma edule	0 (0)	2.61 (1.41)
Corophium volutator	0 (0)	5.91 (3.32)
Nereis diversicolor	4.23 (1.20)	12.85 (7.27)

biodiffusion model in the case of control cores, cores with *Cerastoderma edule* and cores with *Corophium volutator* and the gallery-diffuser model (François et al. 2002) in the case of *Nereis diversicolor* are indicated in Table 5. The percentages of the luminophore distributions explained by the mixing models were 99.9%, 98.0, 92.3, and 59.2% in the control, and in cores with *C. edule*, *C. volutator* and *N. diversicolor*, respectively. A significant difference in biodiffusion intensities was measured among the 4 treatments (1-way ANOVA, $p < 0.05$). The 3 species presented higher biodiffusion transports (mean of 2.61×10^{-3}, 5.91×10^{-3} and 12.85×10^{-3} cm d^{-1} for *C. edule*, *C. volutator* and *N. diversicolor*, respectively) than those measured in the controls (mean of 0.13×10^{-3} cm d^{-1}). The highest sediment biodiffusion was due to *N. diversicolor*. In contrast to the other species, *N. diversicolor* was also responsible for non-local mixing with a mean intensity of 4.23% of tracer d^{-1}.

Flux rates

Water movements from water column to the sediment (measured with bromide) were significantly different among treatments (1-way ANOVA, $F_{(3,8)} = 19.6$, $p < 0.01$, Fig. 3A). The occurrence of *Cerastoderma edule* did not significantly affect the water exchange at the water-sediment interface compared to the control (Scheffé tests, $p > 0.9$). In contrast, *Corophium volutator* and *Nereis diversicolor* tripled water movement compared to the control (Scheffé tests, $p < 0.05$). The same patterns were observed for oxygen flux into the sediment (Fig. 3B, 1-way ANOVA, $F_{(3,8)} = 13.6$, $p < 0.001$). The cores with *C. volutator* or *N. diversicolor* exhibited between 2- and 3-fold higher oxygen uptakes than controls and cores with *C. edule* (Scheffé tests, $p < 0.05$).

The release of ammonium from the sediment depended on the animal (1-way ANOVA, $F_{(3,8)} = 39.8$, $p < 0.001$, Fig. 3C). The 3 species tended to increase the flux of ammonium from the sediment to the water column (example in Fig. 1). However, the increase was not significant with *Cerastoderma edule* despite mean values of ammonium release that were 3-fold higher than in control cores (Scheffé test, $p > 0.05$). The 2 other species significantly increased the flux of ammonium from the sediment to the water column (Scheffé tests, $p < 0.05$). Moreover, *Nereis diversicolor* produced a significantly (Scheffé tests, $p < 0.05$) higher exchange of ammonium from the sediment to the water column than the other treatments.

The results concerning the release of nitrate from the sediment presented a different pattern from ammonium release (Fig. 3D). Significant differences were

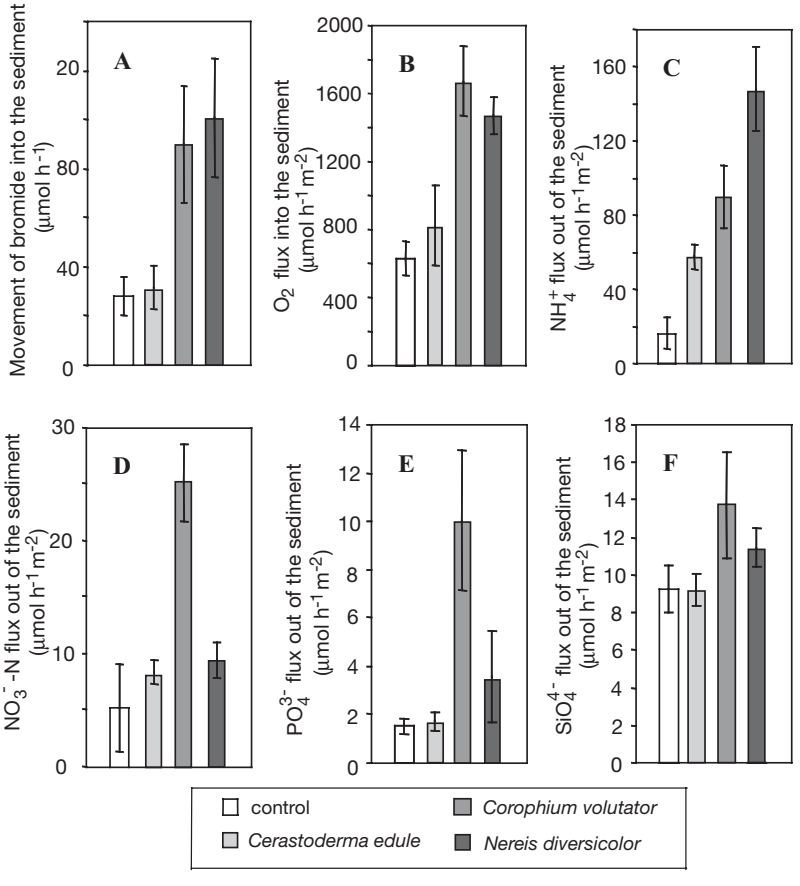


Fig. 3. Fluxes of (A) bromide, (B) oxygen, (C) ammonium, (D) nitrate, (E) phosphate, and (F) silicate across the water-sediment interface for the 4 treatments. Rates are given as mean ± SD of 3 cores

measured among treatments (1-way ANOVA, $F_{(3,8)}$ = 32.0, $p < 0.001$) due to a 2.5- to 5-fold higher release of nitrate with *Corophium volutator* than in other treatments (Scheffé tests, $p < 0.05$). In contrast to *C. volutator*, *Cerastoderma edule* and *Nereis diversicolor* did not significantly (Scheffé tests, $p > 0.3$) affect nitrate release from the sediment. As for nitrate release, phosphate fluxes from the sediment to the water column showed that *C. volutator* significantly increased this process whereas the other species did not affect it (1-way ANOVA, $F_{(3,8)}$ = 15.7, $p < 0.005$, Fig. 3E, Scheffé tests, $p < 0.05$, *C. volutator* versus other treatments). Significant differences were measured among treatments for silicate release (1-way ANOVA, $F_{(3,8)}$ = 4.9, $p < 0.05$, Fig. 3F). However, the statistical tests did not indicate significant differences between pairs of treatments (Scheffé tests, $p > 0.05$ for all comparisons).

Regression analyses showed that biogeochemical fluxes (oxygen, ammonium, phosphate, and silicate fluxes) were linearly linked to water movement from the water column to the sediment in cores (Fig. 4). In contrast, NO_3^- flux out of the sediment was not correlated with water movement. This lack of significant link was due to the fact that *Corophium volutator* and *Nereis diversicolor* produced different NO_3^- fluxes in cores whereas they influenced similarly the water transport (Fig. 3A).

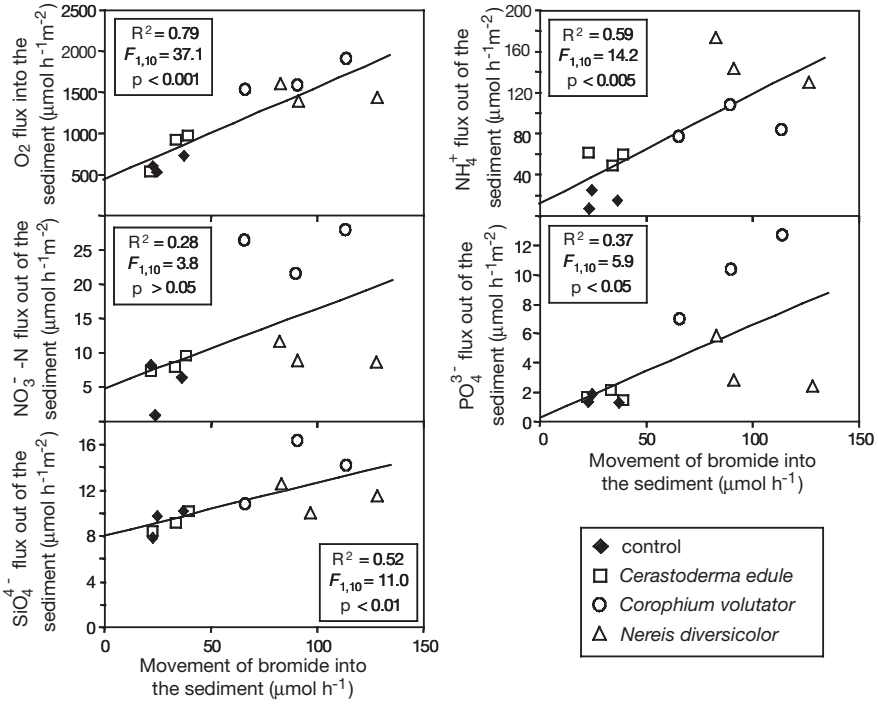


Fig. 4. O_2 and solute fluxes in relation to water movement from the water column to the sediment in the 12 cores. The fitted lines are based on simple regression (n = 12)

Pore water content and chemistry

The porosity of the sediment ranged from 42.5 to 67.6% (expressed as percentage of volume occupied by pore fluid in the wet sediment volume) with a decrease with depth in all cores. Despite the occurrence of biogenic structures and tracks, differences in porosity between inhabited and non-inhabited microcosm were not significant.

In all treatments, the concentration of ammonium increased with depth (RM ANOVA, $F_{(6,48)}$ = 242, $p < 0.001$, depth effect, Fig. 5A). Vertical profiles of ammonium were significantly different among treatments (RM ANOVA, $F_{(3,8)}$ = 15.3, $p < 0.001$, treatment effect). *Cerastoderma edule* and *Corophium volutator* did not affect the concentration of ammonium in pore water compared to the control (Scheffé tests, $p > 0.3$) whereas *Nereis diversicolor* significantly decreased ammonium in pore water (Scheffé test, $p < 0.05$).

Nitrate concentration in pore water peaked in the top first cm of the sediment in all cores except with *Nereis diversicolor* (Fig. 5B). A significant difference in concentration of pore water nitrate was measured among the treatments (RM ANOVA, $F_{(3,8)}$ = 13.1, $p < 0.01$, treatment effect). A significant statistical interaction between treatment and depth effects was measured on nitrate concentrations (RM ANOVA, $F_{(18,48)}$ = 11.0, $p < 0.001$). This statistical result was related to the increase of dissolved nitrate in the top 2 cm of the sediment with *Cerastoderma edule* and *Corophium volutator* compared to the control whereas *Nereis diversicolor* produced a lower concentration of dissolved nitrate in the top first cm of the sediment and increased the concentrations deeper in the cores compared to the control (Fig. 5B).

As for ammonium, phosphate and silicate concentrations in pore water increased with depth (Fig. 5C,D, RM ANOVA, $F_{(6,48)}$ = 147 and 56.9 for phosphate and silicate respectively, $p < 0.001$, depth effect). The concentrations of these 2 compounds in pore water depended on treatments (RM ANOVA, $F_{(3,8)}$ = 20.2 and 6.3 for phosphate and silicate respectively, $p < 0.05$, treatment effects). These significant differences were mainly due to the treatment with *N. diversicolor*. The occurrence of this worm produced lower concentrations of phosphate and silicate in pore water in comparison with the control (Scheffé tests, $p < 0.05$)

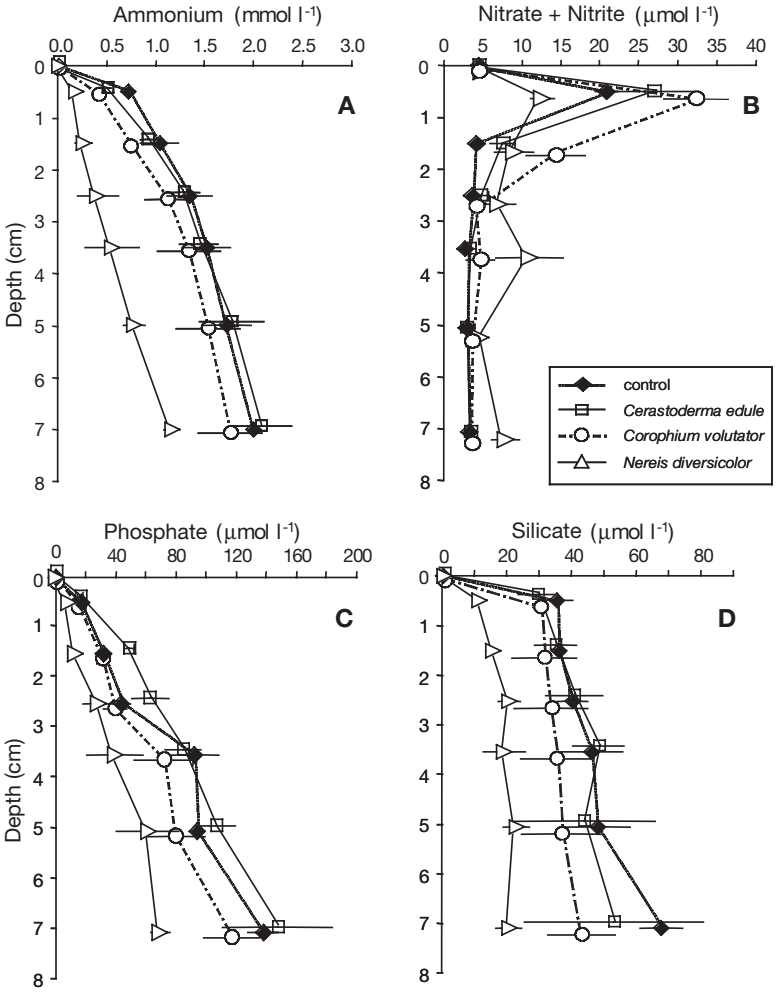


Fig. 5. Vertical profiles of porewater (A) ammonium, (B) nitrate, (C) phosphate, and (D) silicate for the 4 treatments. Error bars are mean ± 1 SD (n = 3 cores)

whereas the other treatments did not significantly affected the pore water concentration of these compounds (Scheffé tests, p > 0.3).

Total organic carbon and total nitrogen

Total organic carbon (TOC) values in the homogenized sediment introduced into cores at the start of the experiment corresponded to 0.8–0.9% of dry mass of sediment. TOC contents decreased with time and all values reported at the end of the experiment ranged from 0.3–0.7% of dry mass. TOC contents varied significantly with depths, showing highest values at the sediment surface and in the deeper layer with *Corophium volutator* (Fig. 6A, RM ANOVA, $F_{(3, 24)} = 11.4$, $p < 0.001$, depth effect). Although *C. volutator* and *Nereis diversicolor* tended to reduce TOC content at the sediment surface in comparison with the control, no significant differences were measured among vertical profiles of TOC obtained from the 4 treatments (RM ANOVA, $F_{(3, 8)} = 0.2$, $p > 0.8$, treatment effect).

Like TOC, total nitrogen (TN) in sediment decreased from 0.23% dry mass at the start of the experiment to 0.03–0.08% on Day 20 (more than 74% of TN was consumed). In all cores, TN varied significantly with depth and the highest values were measured at the sediment surface (Fig. 6B, RM ANOVA, $F_{(3, 24)} = 34.7$, $p < 0.001$, depth effect). As observed with TOC profiles, no significant differences were detected among treatments due to a high variability between replicate cores (Fig. 6B, RM ANOVA, $F_{(3, 8)} = 0.4$, $p > 0.7$, depth effect).

Bacterial characteristics

The total number of bacteria decreased significantly with depth (Fig. 7A, RM ANOVA, $F_{(4, 32)} = 49.4$, $p < 0.001$, depth effect). A significant difference among treatments was observed (RM ANOVA, $F_{(3, 8)} = 5.3$, $p < 0.05$). The occurrence of *Cerastoderma edule* did not affect bacterial abundance in cores compared to the control (Scheffé tests, p > 0.9) whereas

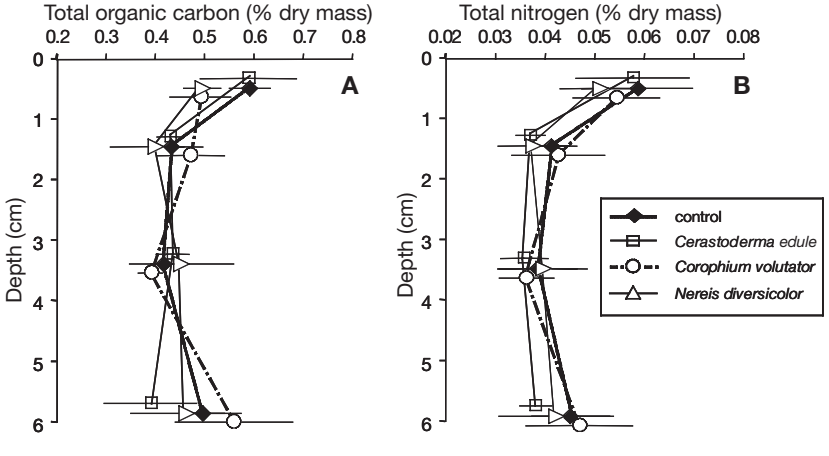


Fig. 6. Vertical profiles of (A) TOC and (B) TN for the 4 treatments. Error bars are mean ± 1 SD (n = 3 cores)

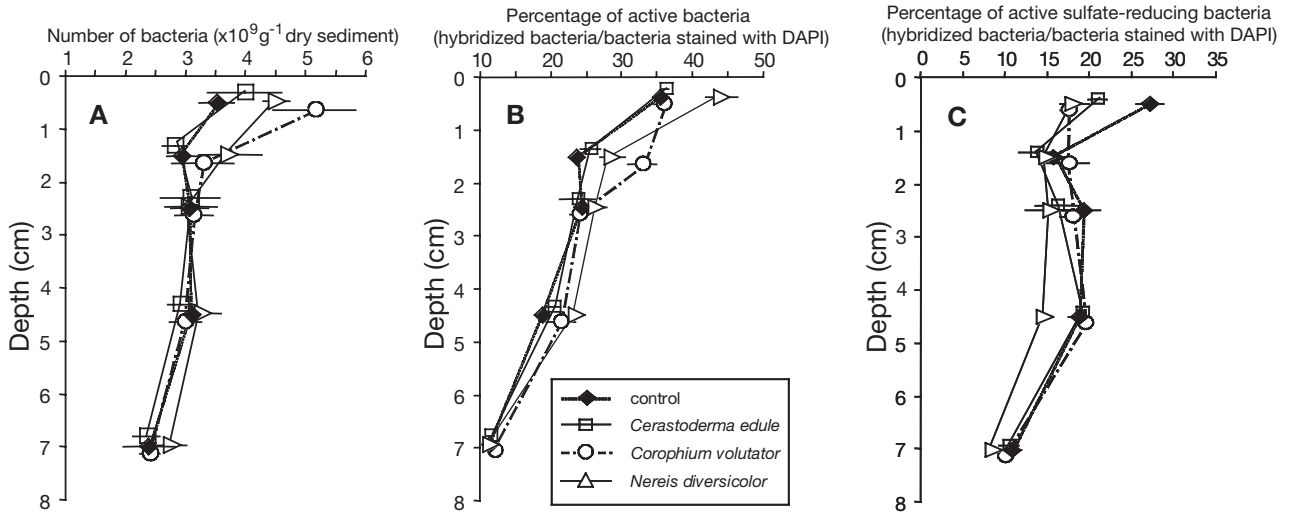


Fig. 7. Vertical profiles of (A) total number of bacteria, (B) percentage of active bacteria, and (C) percentage of active sulphate-reducing bacteria for the 4 treatments. Error bars are mean ± 1 SD (n = 3 cores)

Nereis diversicolor significantly increased the number of bacteria attached to the sediment (Scheffé tests, $p < 0.05$). *Corophium volutator* did not significantly affect the total bacterial abundance of the sediment (Scheffé tests, $p > 0.2$), but increased the number of bacteria by 30% at the sediment surface (Fig. 7A).

The percentage of active bacteria (ratio between bacteria hybridized with EUB and bacteria stained with DAPI) decreased significantly with depth in all cores (Fig. 7B, RM ANOVA, $F_{(4, 32)} = 810$, $p < 0.001$) and was significantly affected by treatments (RM ANOVA, $F_{(3, 8)} = 33.8$, $p < 0.001$). The percentage of active bacteria was not significantly modified by *Cerastoderma edule* compared to the control (Scheffé tests, $p > 0.6$) whereas bacteria were stimulated by the 2 other species (Scheffé tests, $p < 0.01$), and particularly by *Nereis diversicolor* (Scheffé tests, $p < 0.05$).

The percentage of active sulphate-reducing bacteria was also affected in the animal treatments and decreased with depth (Fig. 7C, RM ANOVA, $F_{(3, 8)} = 27.9$ for treatment effect and $F_{(4, 32)} = 92.8$ for depth effect, $p < 0.001$). The percentage of active sulphate reducing bacteria in the sediment was significantly lower in the animal treatments compared to the control (Scheffé tests, $p < 0.01$). With *Nereis diversicolor* present, the percentage of sulphate reducing bacteria was lower than with the other 2 species (Scheffé tests, $p < 0.05$).

DISCUSSION

The present experiment demonstrated that the 3 species exhibited different bioturbation activities. The analysis of sediment reworking indicated that *Cerastoderma edule* and *Corophium volutator* could be classified as biodiffusers whereas *Nereis diversicolor* was classified to the gallery-diffuser group. Biodiffuser animals are known to produce a particle diffusion process throughout all the sediment section reworked by animals whereas gallery-diffusor animals produce a particle diffusion in the layer with very dense gallery systems and a biotransport (non-local transport) at the end of the burrows (François et al. 2002). The classification of *C. volutator* to the biodiffuser group was however surprising in view of the ecology of this species. Because this species produced U-shaped tubes in the sediment, it was classified as a member of the gallery-diffuser group. Thus, our results were due to the spatial resolution of the luminophore experiment. The use of 0.5 cm sediment layers to analyse luminophore transport did not allow a detailed analysis of the sediment from different sections of the burrows because they were only 1 to 2 cm deep. In such conditions, it was not possible to distinguish a possible luminophore peak at the bottom of the burrows. Despite this lack of precision, analyses of sediment reworking clearly showed the vertical distribution of activity by the 3 species. *C. edule* and *C. volutator* were mainly located to the top 2 to 3 cm of the sediment whereas *N. diversicolor* used a greater volume of the sediment (from 0 to 9 cm depth).

The significant relationship between water movement from the water column to the sediment and most solute fluxes demonstrated the importance of the irrigation process induced by animals on biogeochemical processes. Water movement and O_2 uptake, indicative of organic degradation, were greatest in microcosms containing animals living in burrows (*Corophium volutator* and *N. diversicolor*). In contrast, *C. edule*, with its comparatively lower motility, did not affect these pro-

cesses. As supposed by Pelegri & Blackburn (1995), animal irrigation behaviours induced different effects on organic matter mineralization.

Cerastoderma edule pumps water directly from the overlying water by extending its siphons to the sediment surface. As water was transported through the siphons, the pumped water was not directly in contact with the surrounding sediment, limiting the influence of this species on chemical conditions in the sediment. The probable low effect of *C. edule* on O_2 concentrations in the sediment was confirmed by the relatively low modifications of flux rates, pore water chemistry, and microbial characteristics related to this species. The significant impacts of *C. edule* on nitrate concentration in pore water and on sulphate reducing bacteria were exclusively observed in the layer where the bivalve was present (0 to 2 cm depth). At this layer, the percentage of active sulphate reducing bacteria (which are favoured by anaerobic conditions) was reduced by bivalve activity. Activity by *C. edule* transported O_2 into the top sediment, which probably reduced the competitiveness of sulphate-reducing bacteria in comparison with aerobic bacteria. Input of O_2 could also explain the higher concentration of nitrate in the 0 to 2 cm sediment layer compared with the control. As high concentrations of ammonium occurred in the pore water, the presence of O_2 could have stimulated nitrification as a zone of enhanced nitrate appeared at the sediment surface.

In contrast to *Cerastoderma edule*, *Corophium volutator* and *Nereis diversicolor* live in irrigated burrows. Uptake of O_2 and transport of bromide from the water column into the sediment were promoted by these irrigated biogenic structures that increased the exchange surface area between the water column and the sediment. The fauna burrows had significant effects on chemical fluxes and microbial activity. Both *C. volutator* and *N. diversicolor* stimulated the microbial communities as indicated by the greater percentages of EUB-hybridized bacteria in presence of these 2 species and the higher bacterial cell numbers associated with *N. diversicolor*. These stimulatory effects by benthic invertebrates have previously been demonstrated for aerobic bacteria (Hylleberg & Henriksen 1980, Yingst & Rhoads 1980, Reichardt 1988, Pelegri & Blackburn 1994) and were explained as an enhancement of sediment oxidation in the presence of fauna. As observed for *C. edule*, *C. volutator* and *N. diversicolor* reduced the percentages of active sulphate-reducing bacteria. These results could be interpreted by a lower competitive ability of sulphate-reducing bacteria versus aerobic bacteria when transport of O_2 within the sediment is enhanced by invertebrate activities and are in accordance with works of Banta et al. (1999) and Heilskov & Holmer (2001), which showed a reduction of sulphate reduction rates in the upper part of the sediment in presence of macrofauna. They indicated that burrowing and ventilation caused O_2 increase in the sediment, thereby hampering anaerobic metabolism in the upper layer. Our use of *in situ* identification of active microbial cells is consistent with the observations of Banta et al. (1999) and Heilskov & Holmer (2001), and shows that the influence of the macrofauna on microbial communities was linked to inhibition or activation of different groups of bacteria.

The stimulating effect of *Corophium volutator* and *Nereis diversicolor* on microbial activity was largely related to a ventilatory supply of electron acceptors (O_2, NO_3^-) and the removal of reduced metabolites (NH_4^+). However, *N. diversicolor* and *C. volutator* did not produce the same effect on nutrients released from the sediment to the overlying water. *N. diversicolor* created a higher release of reduced compounds (NH_4^+) from the anoxic layers of cores than did *C. volutator*. We suggest that this greater transport of reduced metabolites in the presence of worms is a result of the deeper burrowing of *N. diversicolor* in comparison with *C. volutator*. The pore water chemistry showed that the high effect of *N. diversicolor* on ammonium release from the sediment led to a lower accumulation of this compound in the sediment. As demonstrated by Hansen & Kristensen (1998) and Kristensen & Hansen (1999), the bio-irrigation by *N. diversicolor* increased the exchange of water between the sediment and the water column, reducing the ammonium concentration in the pore water.

Nereis diversicolor also affected concentrations of nitrate, phosphate and silicate in the pore water. However, these results were not correlated with higher release rates of these solutes from the sediment. For phosphate and silicate, it was possible that the lack of relationship between pore water concentration and flux rates resulted from a significant evolution of the system during experiments. The measurement of phosphate and silicate fluxes out of the sediment in control cores indicated an increase by 5-fold of the phosphate flux (0.30 ± 0.11 µmol h^{-1} m^{-2} on Day –1 to 1.53 ± 0.31 µmol h^{-1} m^{-2} on Day 8) and an increase by 2-fold of the silicate flux (4.68 ± 1.02 µmol h^{-1} m^{-2} on Day –1 to 9.24 ± 1.22 µmol h^{-1} m^{-2} on Day 8) during the experiment. Thus, changing fluxes of phosphate and silicate over the course of the experiment made it difficult to compare the fluxes measured on Day 8 with pore water concentrations measured on Day 20. In contrast, the system evolution did not affect ammonium (16.82 ± 3.49 µmol h^{-1} m^{-2} on Day –1 to 16.38 ± 8.51 µmol h^{-1} m^{-2} on Day 8) and nitrate (8.42 ± 1.37 µmol h^{-1} m^{-2} on Day –1 to 5.17 ±3.84 µmol h^{-1} m^{-2} on Day 8) fluxes, allowing the interpretation of pore water concentration profiles obtained on day 20 in parallel with flux rates measured on Day 8.

Concerning nitrate, concentrations in pore water showed small peaks of nitrate at several depths in cores with *Nereis diversicolor*. Such peaks may result from the oxidation of ammonium by nitrifying bacteria in the presence of O_2. The ventilated burrows of *N. diversicolor* might have conducted oxygenated water at depth favouring the nitrification process. Kristensen et al. (1985) showed that the burrow lining is associated with high numbers of nitrifying bacteria. However, the production of nitrate in the sediment was not associated with a high nitrate release from the sediment in presence of *N. diversicolor*. Our results indicated that nitrate concentration in pore water did not vary significantly with depth and suggested that bioirrigation activity of *N. diversicolor* homogenized concentrations in the burrows. In contrast and according to findings of Kristensen (1985), *Corophium volutator* produced a 2.5-fold higher release of nitrate from sediment than did *N. diversicolor*. Sundbäck et al. (2003) also showed the importance of *C. volutator* on nitrate release from the sediment in 2 shallow bays of the west coast of Sweden. As this crustacean was mainly active in the first centimetre of the sediment, it stimulated oxygenation and the associated nitrification process in this zone. During such conditions, the nitrate produced was rapidly exported to the overlying water column whereas nitrate produced in deeper layers of the sediment with *N. diversicolor* might have been more slowly transported to the water column. A balance between nitrification and denitrification is an important determinant of sedimentary DIN exchange (Hansen & Kristensen 1998) and, therefore, the NO_3^- produced in *N. diversicolor* burrows could have been reduced by microbial processes or/and assimilated by bacteria and consequently not been released to the water column. Kristensen (1984) and Kristensen et al. (1991) demonstrated that ventilation of burrows by *Nereis* sp. is intermittent, which gives a complex temporal variability of chemical conditions, enhancing coupled nitrification-denitrification. The ventilation periods would increase nitrate production by O_2 supply into the burrows. However, periods of ventilatory rest would lead to anaerobic conditions that could stimulate the denitrification or the ammonification of the produced nitrate. As a consequence, the production of nitrate during the ventilation of the burrows would not result in a higher flux of nitrate from the sediment to the water column.

According to the higher respiration rates and ammonium fluxes measured in the cores with *Corophium volutator* and *Nereis diversicolor* in comparison with control, it could be assumed that these 2 species would produce a significantly higher reduction of sediment TOC and TN than in the control. However, our data do not support this assumption; only a weak tendency indicated that *C. volutator* and *N. diversicolor* caused some reduction of TOC in the upper sediment layer. The lack of significant differences among treatments on TN may result from the strong consumption of TN in all cores (more than 74%), reducing the ability to detect treatment differences. The lack of clear effects of animal treatments on TN and TOC consumption was linked to the complex influence of animal burrowing on the micro-distribution of the particulate organic matter in the sediment (Kristensen et al. 1985, de Vaugelas & Buscail 1990) because bioturbation activities may increase (by sequestration of organic matter or/and stimulation of the bacterial assimilation of dissolved nutrients present in the overlying water) or reduce (by stimulation of organic matter processing) the concentration of C and N in the sediment. Finally, it seems that sampling of TOC and TN need to be more detailed (e.g. assessment of C and N contents in burrows or/and at different times of the experiment) in order to better describe the impact of different functional groups of invertebrates on TOC and TN in the sediment.

In the Gullmarsfjord, shallow water communities are dominated by the 3 species studied in the present work. According to our results, *Nereis diversicolor* produced the greatest organic matter processing, probably related to its deep activity from the surface to 9 cm in the sediment. *Corophium volutator* also stimulated the biogeochemical processes but its influence was limited to the top 2 cm of the sediment. *Cerastoderma edule* had no strong influence on processes in the sediment. However, this low effect of the bivalve might result from the objectives of the study which only focused on the sedimentary processes. In these conditions, benthic invertebrates feeding on organic matter in the sediment (*C. volutator* and *N. diversicolor*) had logically a higher impact than a strict suspension feeder (*C. edule*). The presence of an active suspension feeder will influence the particulate organic matter of the near-bottom water and biodeposition on the sediment surface (see review of Graf & Rosenberg 1997). Many studies (Officer et al. 1982, Loo & Rosenberg 1989, Graf 1992) have demonstrated that filter feeding has a strong effect on phytoplankton biomass and production of faecal pellets (due to biodeposition) in shallow water areas. Thus, a study integrating both pelagic and benthic processes may emphasize that *C. edule* affects the whole ecosystem functioning by linking the pelagic and the benthic environments.

The present work demonstrated that different influences of benthic species on the physical habitat through their specific bioturbation activities (sediment reworking, biogenic structure building, bioirrigation) created different effects on the microbial communities

and biogeochemical processes in the sediment. The divergent effects of *C. volutator* and *N. diversicolor* suggest that it is not adequate to simply classify animals as deposit feeders without consideration of ingestion-digestion mechanisms, morphologies, and behaviours (see an example with bivalves in Grémare et al. 2004). Several authors (e.g. Fauchald & Jumars 1979, Pearson & Rosenberg 1987, Pearson 2001, Rosenberg 2001) developed classifications of benthic invertebrates that advocated a subdivision of the trophic functional groups traditionally recognized in the benthos (herbivores, suspension feeders, detritus feeders, carnivores, and omnivores) that relate to detailed information available on the feeding behaviour and morphology of the species. The classification of Pearson & Rosenberg (1987) using 4 modes of functional traits (feeding mode, mobility mode, degree of mobility, mode of feeding habit) aimed to link marine benthic communities and benthic habitats. However, as bioturbation activities are related to the functional traits of benthic animals, we need to develop foraging- and feeding-type perspectives as broad as those used by Pearson & Rosenberg (1987) to classify benthic invertebrates among bioturbation functional groups in marine habitats.

Acknowledgements. This study was supported by a post-doctoral fellowship to F.M.-B. sponsored by Munkedal A.B. (Munkedal, Sweden) in association with the Kristineberg Marine Research Station. We thank S. Eriksson, M. Kuylenstierna, A.-C. Ask, and the staff of the Kristineberg Marine Research Station for their help during laboratory work. We are grateful to S Hulth (Analytical and Marine Chemistry, Department of Chemistry, Göetborg University) for use of his equipment for microcosm experiments. We are also grateful to the 2 referees for their valuable criticisms and corrections of the manuscript.

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Editorial responsibility: Kevin Carman, Baton Rouge, Louisiana, USA

Submitted: December 20, 2003; Accepted: June 3, 2004
Proofs received from author(s): August 3, 2004