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Florian Mermillod-Blondin[a,*], Frédérique François-Carcaillet[b,1], Rutger Rosenberg[c]

[a]*UMR-CNRS 5023, Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon I, Domaine Scientifique de la Doua, bâtiment Forel, 69622 Villeurbanne Cedex, France*

[b]*UMR-CNRS 7621, Laboratoire d'Océanographie Biologique de Banyuls, Université Paris VI, BP 44 Banyuls-sur-mer Cedex, France*

[c]*Department of Marine Ecology, Göteborg University, Kristineberg Marine Research Station, 45034 Fiskebäckskil, Sweden*

The main objective of this study was to measure the impact of benthic invertebrate diversity on processes occurring at the water–sediment interface. We analyzed the effects of interactions between three shallow water species (*Cerastoderma edule*, *Corophium volutator*, and *Nereis diversicolor*). The impacts of different species richness treatments were measured on sediment reworking, bacterial characteristics, and biogeochemical processes (bromide fluxes, O_2 uptake, nutrient fluxes, and porewater chemistry) in sediment cores. The results showed that the three species exhibited different bioturbation activities in the experimental system: *C. edule* acted as a biodiffusor, mixing particles in the top 2 cm of the sediments; *C. volutator* produced and irrigated U-shaped tubes in the top 2 cm of the sediments; and *N. diversicolor* produced and irrigated burrow galleries in the whole sediment cores. *C. edule* had minor effects on biogeochemical processes, whereas the other species, through their irrigation of the burrows, increased the solute exchange between the water column and the sediment two-fold. These impacts on sediment structure and solute transport increased the O_2 consumption and the release of nutrients from sediments. As *N. diversicolor* burrowed deeper in the sediment than *C. volutator*, it irrigated a greater volume of sediments, with great impact on the sediment cores.

Most treatments with a mixture of species indicated that observed values were often lower than predicted values from the addition of the individual effects of each species, demonstrating a negative interaction among species. This type of negative interaction measured between species on ecosystem processes certainly resulted from an overlap of bioturbation activities among the three species which lived and foraged in the same habitat (water–sediment interface). All treatments with *N. diversicolor* (in isolation and in mixture) produced similar effect on sediment reworking, water fluxes, nutrient releases, porewater chemistry, and bacterial characteristics. Whichever species associated with *N. diversicolor*, the bioturbation activities of the worm hid the effect of the other species. The results suggest that, in the presence of several species that use and modify the same sediment space, impact of invertebrates on ecosystem processes was essentially due to the most efficient bioturbator of

* Corresponding author. Tel.: +33 4 72 43 13 64; fax: +33 4 72 43 15 23.

E-mail address: mermillo@univ-lyon1.fr (F. Mermillod-Blondin).

[1] Present address: UMR-CNRS 5119 "Ecosystèmes lagunaires", Université Montpellier II, Case 093, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France.

the community (*N. diversicolor*). In consequence, the functional traits (mode of bioturbation, depth of burrowing, feeding behaviour) of an individual species in a community could be more important than species richness for some ecosystem processes.

Keywords: *Nereis diversicolor*; *Corophium volutator*; *Cerastoderma edule*; Functional diversity; Bioturbation; Microbial activity; Organic matter processing

1. Introduction

The relationship between biodiversity and ecosystem functioning is of great interest in order to describe the potential effect of biodiversity loss on ecosystem processes (Naeem et al., 1994; Tilman et al., 1997; Loreau et al., 2001). The impact of biodiversity on ecosystem processes depends not only on the functional characteristics of each species but also on the interactions among species present in the community (Loreau et al., 2001; Cardinale et al., 2002; Jonsson and Malmqvist, 2003a). In marine ecosystems, the benthic biodiversity–ecosystem functioning relationship has recently been studied in shallow water habitats (Emmerson and Raffaelli, 2000; Emmerson et al., 2001; Raffaelli et al., 2003; Biles et al., 2003). These studies showed that the relationship between invertebrate biodiversity and ecosystem function was idiosyncratic (Emmerson et al., 2001): species diversity affects ecosystem function but not in a predictable direction (Lawton, 1994). They also demonstrated that the diversity of functional traits (e.g. mode of bioturbation) strongly affects the measured ecosystem process, suggesting that functional traits may be at least as important as species richness on ecosystem functioning. However, these studies focused on only one process which was the ammonium release from sediments. The ammonium release is, however, an effect of several processes in the sediment (Raffaelli et al., 2003): stimulation of microbial mineralization of organic nitrogen, alteration of the conversion rates of ammonium to nitrate (nitrification), and modification of the transport rate of ammonia to the surface through bioirrigation. Therefore, it is needed to take into account several infaunal activities in order to better understand the role of biodiversity in shallow water sediments.

In a previous study, Mermillod-Blondin et al. (2004) demonstrated that three species dominant of the shallow water area in the Skagerrak (*Cerastoderma edule*, *Corophium volutator*, and *Nereis diversicolor*) have distinct effect on water–sediment processes and characteristics (sediment reworking, O_2 uptake, nutrient release from sediments, bacterial communities). Based on these first results testing the role of isolated species at the water–sediment interface, the influence of multi-species treatments was measured in the present study to determine the importance of the interactions between the three species on the ecosystem processes. An experimental approach was developed to quantify the influence of different species treatments on several ecosystem processes. Complementary to the previous studies summarized in the paper of Raffaelli et al. (2003), the aim of the present study was to determine if the interactions among species of a community have an impact on ecosystem processes. We compared the impacts of simple assemblages of species (ranging from 1 to 3 species) on a wide range of processes (bioturbation, dissolved oxygen uptake, water fluxes, nutrient fluxes) and system characteristics (porewater chemistry, bacterial characteristics) in order to detect most impacts of invertebrates in sediments.

2. Materials and methods

2.1. Sediment and animal collection

Sediment was collected in June 2002 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 silt-sand sediment (60% of fine sand and 40% of silt) mixed with shell debris, and with an organic content varying between 0.8% and 1.7% (Pihl, 1986).

To reduce the natural heterogeneity and to obtain equal starting conditions, sediment was homogenized before use. Sample from the upper 8–10 cm of sediments was sieved through a 1-mm mesh to remove macrofauna and larger particles (shells). Intact specimens of *C. edule* (0.9 to 1.1 g wet weight without the shell, 30 mm long), *C. volutator* (0.025 to 0.035 g wet weight, 5 to 8 mm long), and *N. diversicolor* (0.2 to 0.4 g wet weight, 6 to 10 cm long) were collected in the Gullmarsfjord. These three species were selected because they were the dominating species of the shallow area in the Gullmarsjford. They also produce different effects in the sediment. *C. edule* is a suspension feeder usually found at 2–4 cm depth in the sediment. It occurs commonly in densities of 15–300 ind. m^{-2} (Muus, 1967; Rasmussen, 1973) but higher densities of juveniles (6000–14,000 ind. m^{-2}) have been reported in the Skagerrak (Möller and Rosenberg, 1983). *C. volutator* lives in irrigated U-shaped burrows at 2 to 4 cm depth in the sediment. The mean densities of *C. volutator* can be up to 12,000 ind. m^{-2} the Skagerrak (Möller and Rosenberg, 1982). *N. diversicolor* lives in mucus-lined gallery of burrows extending 6 to 12 cm into sediment (Davey, 1994). *N. diversicolor* can act both as deposit-feeder and as filter-feeder (Riisgard, 1991). *N. diversicolor* is common in shallow waters and has been found in numbers of 500 to 5000 ind. m^{-2} (Möller, 1985; Vedel and Riisgard, 1993).

2.2. Experimental setup

Sediment cores were established by transferring the homogenized sediment into Plexiglas® tubes (28-cm long and 10-cm internal diameter) to a depth of ≈18 cm. Twenty four cores were placed in a dark room at 14 °C. A continuous in situ water (salinity: 32 psu, controlled temperature: 14 °C) flow system (120 ml min^{-1}) was installed. The turnover rate of the overlying water (10 cm deep) was between 6 and 7 min. The macrofauna was introduced in the microcosms 10 days after the sediment installation to permit a recovery from the disturbance due to sampling and core preparation.

Eight treatments were performed with three replicate cores per treatment with the following number of individuals per core: (1) without macrofauna (control), (2) 2 *C. edule* (Ce), (3) 40 *C. volutator* (Cv), (4) 5 *N. diversicolor* (Nd), (5) 2 *C. edule* and 40 *C. volutator*, (6) 2 *C. edule* and 5 *N. diversicolor*, (7) 40 *C. volutator* and 5 *N. diversicolor*, and (8) 2 *C. edule*, 40 *C. volutator*, and 5 *N. diversicolor*. The macrofaunal densities tested were equivalent to 250, 5100, and 640 ind. m^{-2} for *C. edule*, *C. volutator*, and *N. diversicolor*, respectively. The densities tested for each species in isolation were close to average densities of natural sediments observed in the Skagerrak (see above). In mixture treatments, the densities tested were probably higher than those reported from a single site for the three species but these densities gave a range of biomass (from 0 to 5.5 g) that was comparable to those (from 0 to 8 g) tested by Emmerson et al. (2001) in the same area. Chemical measurements, before the faunal introduction, confirmed that O_2 uptake (935±62 μmol h^{-1} m^{-2}, mean±SD), ammonium (17.51±4.20 μmol h^{-1} m^{-2}), nitrate (8.05±1.22 μmol h^{-1} m^{-2}), and phosphate (0.29±0.11 μmol h^{-1} m^{-2}) releases from the sediment were comparable in the 24 cores. The animals dug rapidly (<2 h) into sediments except four individuals of *C. edule* that moved at the sediment surface and took more time (from 1 to 3 days) to dig into the sediment matrix. The experiment was run with animals for 20 days in July 2002, at the Kristineberg Marine Research Station (KMRS).

During the 20 days of the experiment, physical, chemical and bacteriological parameters were measured in the cores to quantify the impact of macrofauna. Exchanges of DIN (dissolved inorganic nitrogen; NO_2^-+NO_3^- and NH_4^+), and phosphate (PO_4^{3-}) were measured at day 8 of the experiment. Water–sediment exchange was measured on day 10 of experiment using bromide as tracer of water. O_2 consumption within cores was estimated after 12 days of 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. To determine the impact of invertebrates on microbial community, labeled rRNA-targeted nucleic acid probes were used to identity active microbial cells in their natural habitats (Amann et al., 1997). Surface particle redistribution, porewater chemistry (NO_2^-+NO_3^-, NH_4^+, and PO_4^{3-}), nitrogen and organic carbon content of the sediment were analyzed at the end of the experiment.

2.3. Chemical and bacterial measurements

2.3.1. Benthic oxygen uptake

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 were determined from changes over time of the concentration of O_2 in the water column (Kristensen and Hansen, 1999). Tests showed that oxygen decrease never exceeded 20% of the initial oxygen concentration. O_2 concentrations of the water samples were analyzed within 1 h by the Winkler titration technique.

2.3.2. Nutrient exchange fluxes

In each core, the overlying water was mixed using a magnetic stirring (60 rpm). The cores were then incubated during 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_2^-+NO_3^- and phosphate across the sediment–water interface were calculated from changes over time in the concentration of each species in the water column.

2.3.3. Water–sediment fluxes

Bromide ($[Br^-]=16\pm0.35$ 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 flux 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).

2.3.4. Sediment reworking

Sediment reworking in the cores was quantified by the luminophore tracer technique (Mahaut and Graf, 1987; Gerino, 1990). On day 0 of the experiment, 1 g of 100–150 μm luminophores (natural sediment particles dyed with fluorescent paint) was deposited on the sediment surface after the invertebrate introduction. At the end of the experiment (20 days), water was removed, cores were opened, and sediment and luminophores were sampled. The top 9 cm of each core was collected by layer of 0.5 cm. Each layer was homogenized and a 1-g sub-sample was dried at 50 °C for luminophore counting. The numbers of luminophores were estimated under U.V. light microscope and converted into g of tracers per g of dry sediment. The impact of invertebrates on particulate matter mixing was quantified thanks to 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 day^{-1}.

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 two parameters, a biodiffusion rate (expressed in day^{-1}) and a non-local transport rate (expressed in day^{-1}). We used the following transformation to make the biodiffusion rate independent of the cell size and to express it as it appeared in the literature:

$$Db = R_{Db}\delta^2$$

where Db is the biodiffusion coefficient (cm^2 day^{-1}), R_{Db} is the biodiffusion rate as used in our models (day^{-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 by day (% day^{-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).

2.3.5. Porewater and sediment chemistry

During core opening, sub-samples were taken as for the luminophore analyses and used for determination of porewater content (weight loss after 24 h at 105 °C) and analysis of the porewater chemistry. Sediment was collected in each core for six layers: 0–1, 1–2, 2–3, 3–4, 4–6, and 6–8 cm. Porewater was obtained by centrifugation (30 min at 5000 rpm). The supernatant (5 ml) was collected and frozen (−20 °C). NO_2^-+NO_3^-, NH_4^+, and PO_4^{3-} concentrations in pore were measured by standard methods with a TRAACS 800, four-channel automized nutrient analyzer (Grasshoff et al., 1983). The remaining centrifuged sediment was dried at 60 °C over night and used to measure TOC and N on a NA 1500 NC Carlo Erba elemental analyzer (Fisons) according to Hedges and Stern (1983).

2.3.6. Bacterial measurements

For each sleeved plexi-core (30 mm in diameter) sampled on day 15, 2 g of wet sediment was immediately collected at five 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 milliliters of 0.1% pyrophosphate in PBS was 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 during 160 s with 50% of active cycle. All suspensions were finally supplemented with the detergent NP-40 (Flucka, Buchs, Switzerland) 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, Buchs, Switzerland) according to Schönholzer et al. (2002). Probes were used to detect the Domains Bacteria (probe EUB 338, eubacteria) and bacteria responsible of 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 (Citifluor, London, UK) 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.

2.4. Statistical analyses

2.4.1. Effect of each species treatment

The rates of sediment reworking obtained from the eight treatments were ln(x+1) transformed to homogenize variances. Afterwards, differences in rates of sediment reworking were tested using a one-way analysis of variance (ANOVA). Tuckey HSD post hoc tests were performed if significance was detected to determine which treatment differed. Flux measurements (O_2 consumption, water exchange, nutrient releases from the sediment to the water column) were compared among treatments using a one-way analysis of variance. In the analyses, the total numbers of bacteria were log transformed. The effects of animals on porewater chemistry and microbial variables were compared using a two-way ANOVA with treatment and depth as main effects (Statistica 5 TM, Statsoft, Tulsa). Scheffé post hoc tests were performed to determine which treatment differed.

2.4.2. Estimation of interactions between species

According to the statistical designs used in the biodiversity-functioning studies in marine sediment (Emmerson and Raffaelli, 2000; Emmerson et al., 2001; Raffaelli et al., 2003; Biles et al., 2003), the influence of diversity on ecosystem processes can be analyzed using two methods: (i) use of a set of biomasses or a constant biomass for different diversity treatments and (ii) use of an additive model which does not require a control of the biomass. Although the first method was the most adequate to determine

the global relationship between biodiversity and ecosystem functioning using a random set of diversity treatments, the second method was well adapted to specifically analyse the influence of the interactions between the species tested. According to the objectives, we used this second method (additive model, Raffaelli et al., 2003, p. 137) based on single-species effects against which we compare observed multi-species effects. The comparison between the observed effects with two- or three-species mixtures and the predicted effects of mixtures calculated by all combinations of the effects of the species in isolation (individual effects) was used to determine the significance of interaction among species on processes. The prediction is defined as the additive effect of the several species forming the mixture. For example, in the case of the Ce+Cv-treatment, we had:

Predicted values calculated using the additive model
$(n = 9,\ 3 \text{ columns} \times 3 \text{ columns})$
$= E(\text{Ce})_{n=3} + E(\text{Cv})_{n=3}$
were compared with observed values $(n = 3)$
$= E(\text{Ce} + \text{Cv})_{n=3}$

where E(Ce) and E(Cv) are the effects of *C. edule* and *C. volutator* in one-taxon treatments and *E*(Ce+Cv) is the value measured in the mixture of Ce and Cv. With this method, we expected that, in the absence of interactions, the effects of 2 Ce and the effects of 40 Cv would be simply added in the treatment with 2 Ce+40 Cv. Using this additive model, we assumed that effects measured in mixtures were linked to interaction between species rather than a simple effect of animal density (biomass). Because the impact of each species in sediment was linked to its life mode (Mermillod-Blondin et al., 2004), the type of effect (production of burrows, sediment reworking mode) produced by a species in sediment was not expected to be strongly affected by its density. For vertical profiles of solutes, carbon and nitrogen in the sediment, and microbial variables, observed and predicted values were compared using a two-way ANOVA with observed-predicted and depth as main effects. For coefficients of sediment reworking and fluxes, observed and predicted values were compared using Student's *t*-tests. A significant (negative or positive) difference indicates that interactions between taxa modified the system functioning because the effects of the two or three taxa were not simply added in mixture treatments. In addition, it could be observed that the dominant effect of one taxon in mixtures can lead to the detection of significant interactions. Thus, the effects of mixtures were compared with the effects of each taxon forming the mixture to determine if one taxon present in the mixture drove the system functioning. Statistical analyses used to compare mixture effects and one-taxon effects were included in the comparisons among species treatments (Scheffé post hoc tests).

3. Results

3.1. Visual observations

In the control cores, a brownish coloured zone of 12–16 mm depth was observed in the top sediment. Below this oxidized zone, the sediment was grey to greyish-black. In the bioturbated cores, biogenic structures produced by *C. volutator* and *N. diversicolor* extended the oxidized zone into the reduced sediment in the form of 2- to 5-mm-thick oxidized wall linings around the burrows. At the sectioning of cores, burrows were observed down to 2–3 and 8–9 cm depth in treatments with *C. volutator* and *N. diversicolor*, respectively. The presence of *C. edule* extended the oxidized zone in the areas where the bivalves had burrowed. No difference in vertical distribution of each species was noted between one-species treatments and mixtures.

3.2. Effects of species treatments

The luminophore profiles obtained at the end of the experiments showed that the average percentage of tracer measured at the sediment surface (layer: 0–0.5 cm) was the highest in the control cores (98%) and varied from 83% to 26% with invertebrates (Fig. 1). In cores without *N. diversicolor*, the profiles were characterized by a rapid decrease of tracer with depth, indicating a biodiffusive reworking of sediment. With *N. diversicolor*, small peaks of tracer were observed at depth, revealing a non-local transport (transport by fall of the surface sediment in burrows).

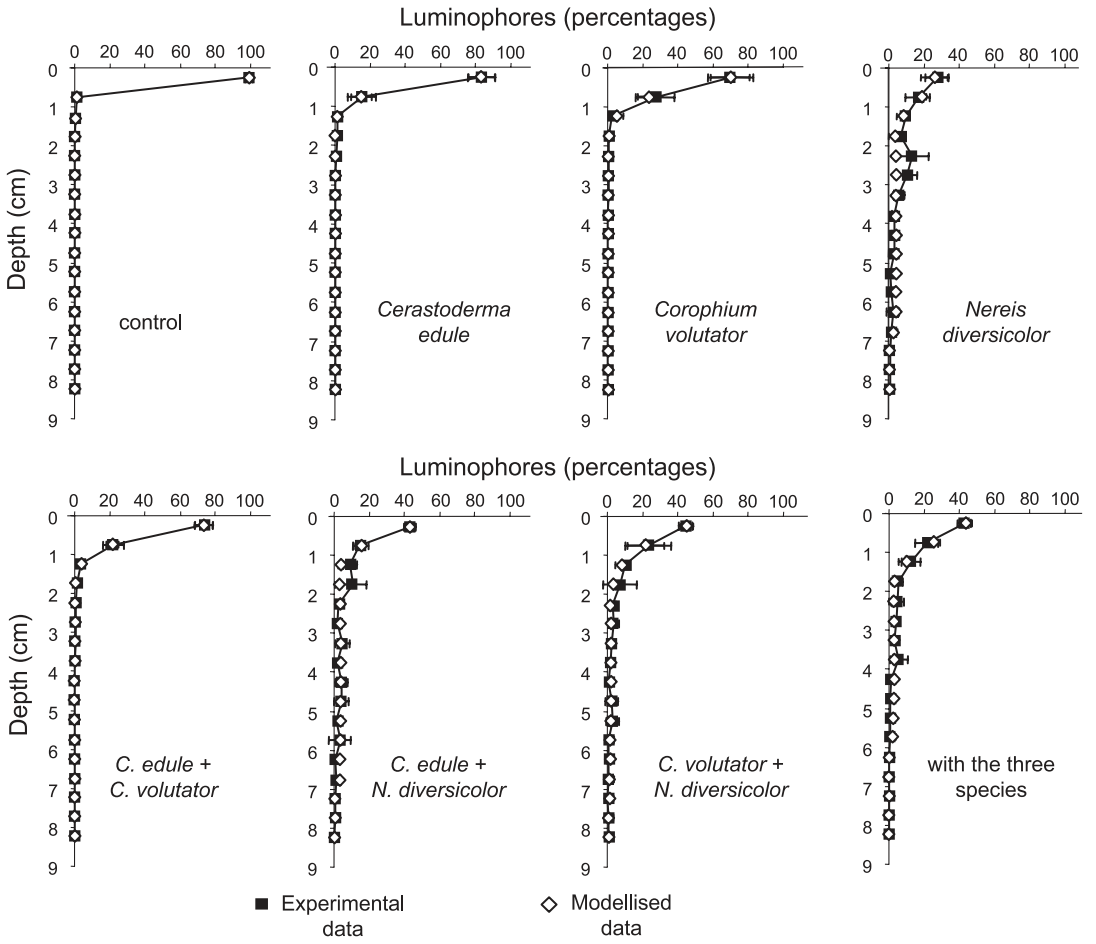

Fig. 1. Depth profiles of luminophores for the eight treatments (means±S.D., n=3). Model profiles were indicated for each treatment.

Modellized data fitted well with experimental data (Fig. 1). The sediment reworking rates were estimated with a biodiffusion model in the case of control cores and cores with *C. edule* and/or *C. volutator* whereas the gallery-diffuser model was used to analyze the cores containing *N. diversicolor*. A significant difference in biodiffusion rates was measured among the eight treatments (one-way ANOVA, $p<0.05$). The seven animal treatments produced higher biodiffusion transports than those measured in the controls (Fig. 2). The highest mean sediment biodiffusion rate was due to the treatment with *N. diversicolor*, but no significant differences between biodiffusion rates were measured among the seven animal treatments. In cores with *N. diversicolor*, a non-local transport was measured and showed that treatment with *N. diversicolor* alone produced a greater effect on this mixing process than mixtures with *N. diversicolor* and the other species (Fig. 2).

All animal treatments significantly increased benthic O_2 uptake and water exchange between overlying water and the sediment (estimated with bromide) in comparison with the control and the *C. edule*-treatment (Fig. 3, one-way ANOVAs, $p<0.001$). Tukey HSD tests ($p>0.2$) indicated that *C. volutator*, *N. diversicolor*, and all mixture-treatments produced a similar effect on benthic O_2 uptake (values ranging from 1400 to 1700 μmol of O_2 consumed h^{-1} m^{-2}) and bioirrigation (values ranging from 90 to 130 μmol of Br^- exchanged h^{-1} m^{-2}). The release of ammonium from the sediment was related to the animal treatments (one-way ANOVA, $p<0.001$, Fig. 4). All animal

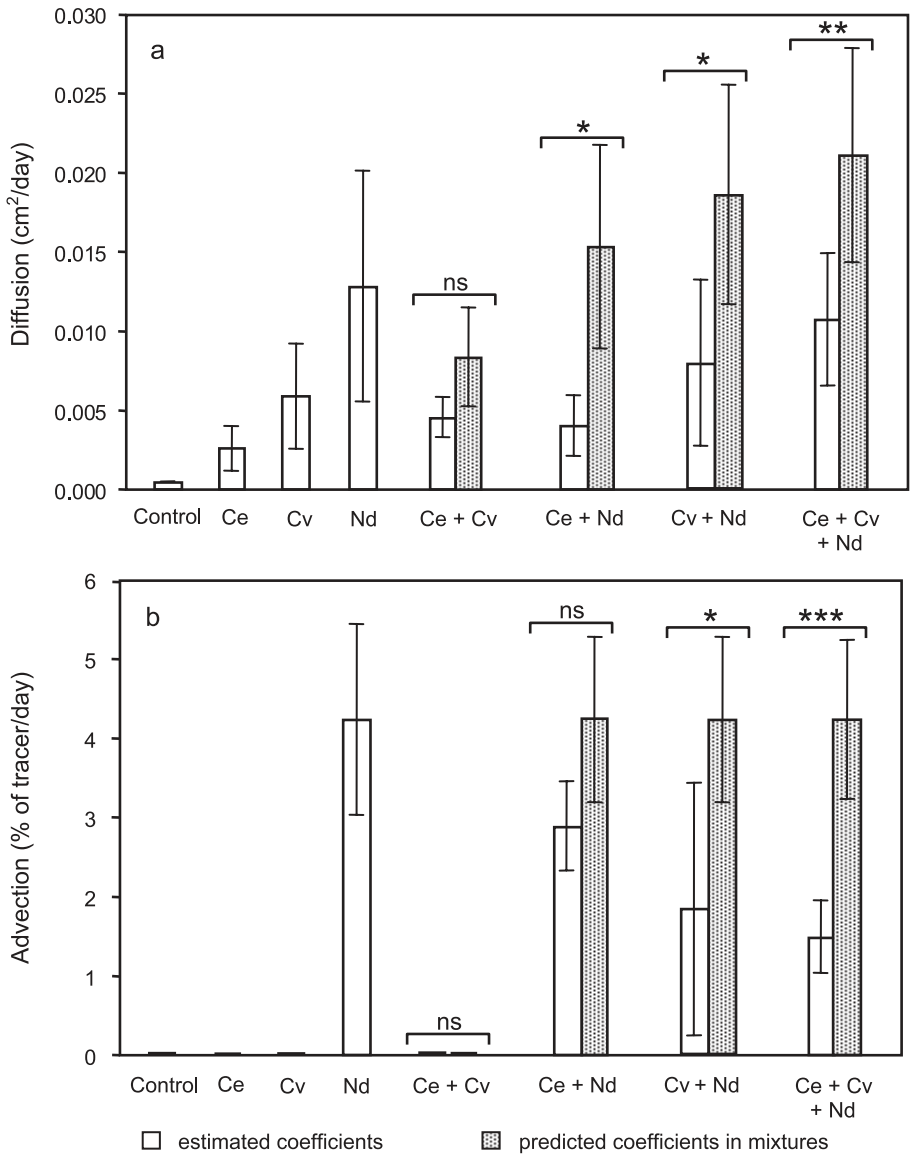

Fig. 2. Diffusion (a) and non-local transport (b) coefficients estimated for the eight treatments (means±S.D., n=3). Predicted coefficients are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (obs-pred, ***p<0.001, **p<0.01, *p<0.05, ns: no significant difference). Animal treatments: Ce: *C. edule*, Cv: *C. volutator*, Nd: *N. diversicolor*, Ce+Cv: *C. edule*+*C. volutator*, Ce+Nd: *C. edule*+*N. diversicolor*, Cv+Nd: *C. volutator*+*N. diversicolor*, and Ce+Cv+Nd: *C. edule*+*C. volutator*+*N. diversicolor*.

treatments increased the flux of ammonium from the sediment to the water column. The *N. diversicolor*-treatment produced a higher exchange of ammonium from the sediment to the water column than other one-species treatments. Addition of species to the *N. diversicolor* treatment did not increase the ammonium release because no significant differences were measured for this process between Nd treatment and Ce+Nd-, Cv+Nd-, and Ce+Cv+Nd-treatments. The release of nitrate and phosphate from the sediment depended on animal treatments (one-way ANOVAs, p<0.001, Fig. 4). The Cv-treatment significantly

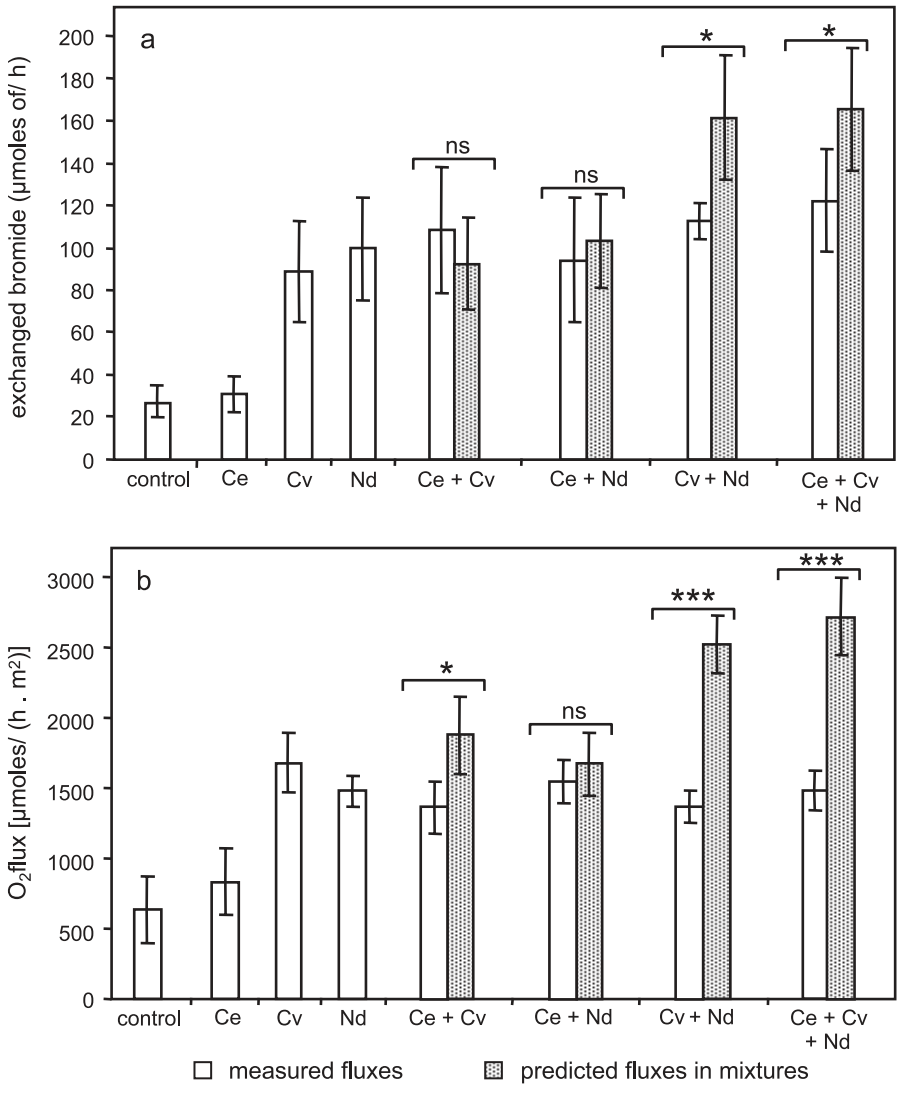

Fig. 3. Fluxes of bromide (a) and oxygen (b) across the water–sediment interface for the eight treatments. Rates are given as mean±S.D. of three cores. Predicted fluxes are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, *p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

increased the release of nitrate and phosphate from the sediment whereas other one-species treatments did not have any such effect. Similar release rates were observed in Cv- and Ce+Cv-treatments (Tukey HSD tests, p>0.6). In contrast, lower nitrate releases from the sediment were measured in other mixture treatments containing *N. diversicolor* (Tukey HSD test, p<0.05).

In all treatments, the concentration of ammonium and phosphate increased in the porewater with depth (two-way ANOVA, p<0.001, depth effect, Figs. 5 and 6). Vertical profiles of ammonium and phosphate were significantly different among treatments (two-way ANOVA, p<0.001, treatment effect). *C. edule* and *C. volutator* did not significantly affect the concentrations of ammonium and phosphate in the porewater compared to the control (Tukey HSD test, p>0.6), whereas the other treatments significantly decreased ammonium in porewater (Tukey HSD tests, p<0.01). The highest

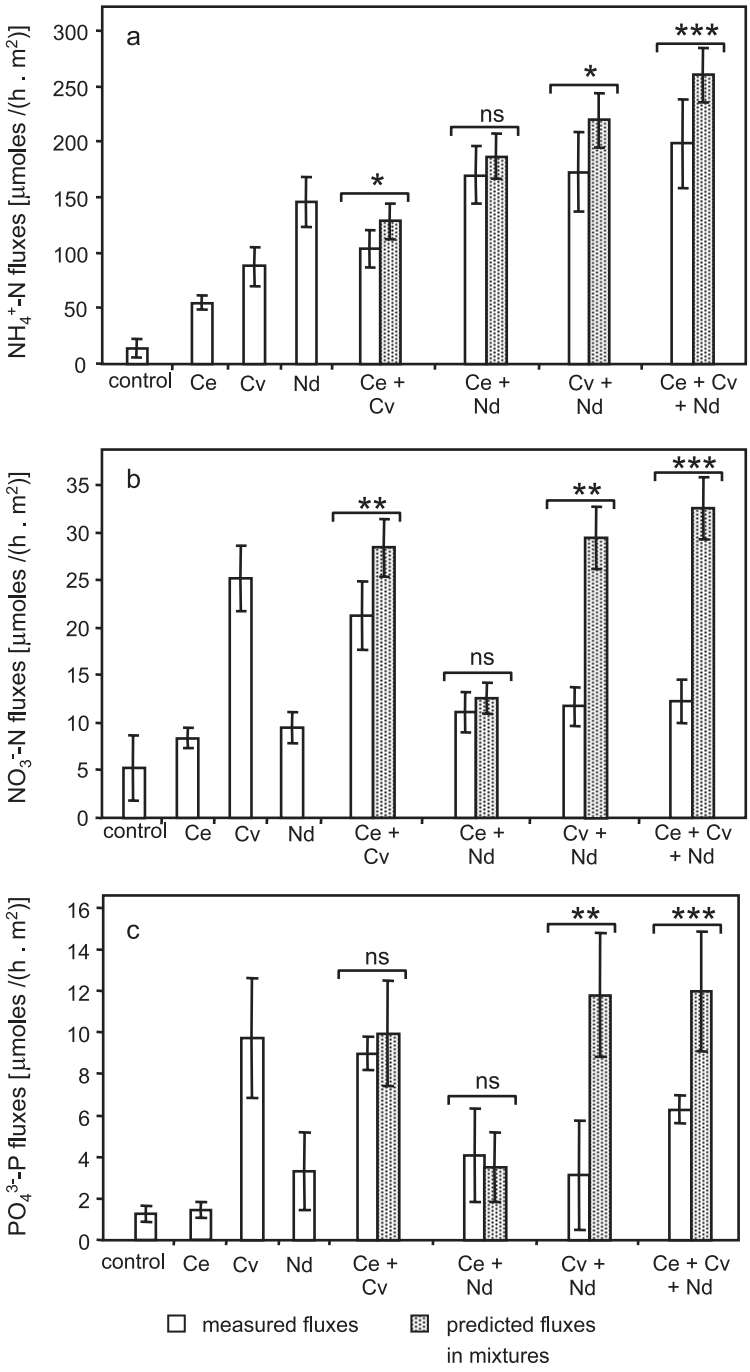

Fig. 4. Fluxes of ammonium (a), nitrate (b), and phosphate (c) across the water–sediment interface for the eight treatments. Rates are given as mean±S.D. of three cores. Predicted fluxes are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, **p<0.01, *p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

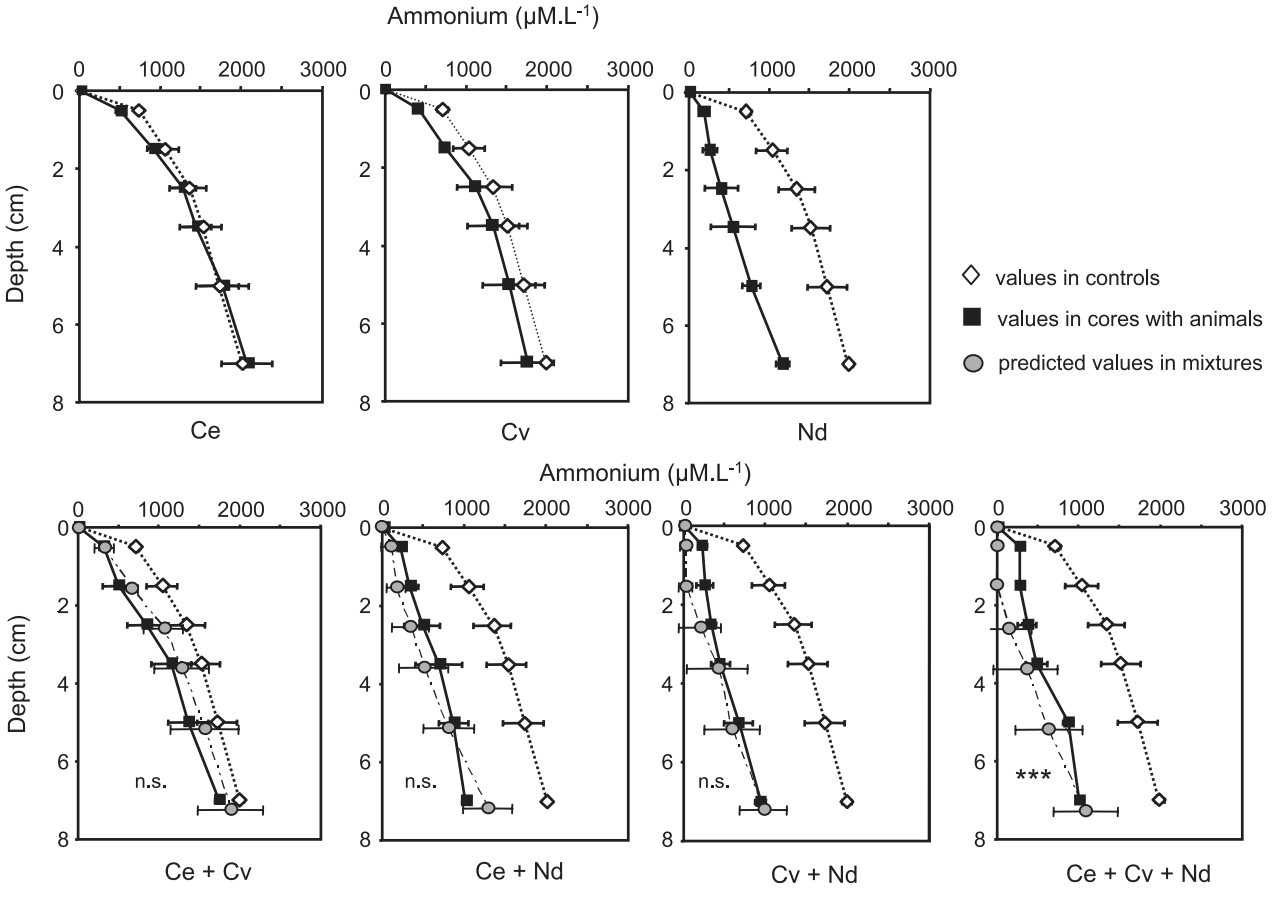

Fig. 5. Vertical profiles of porewater ammonium for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

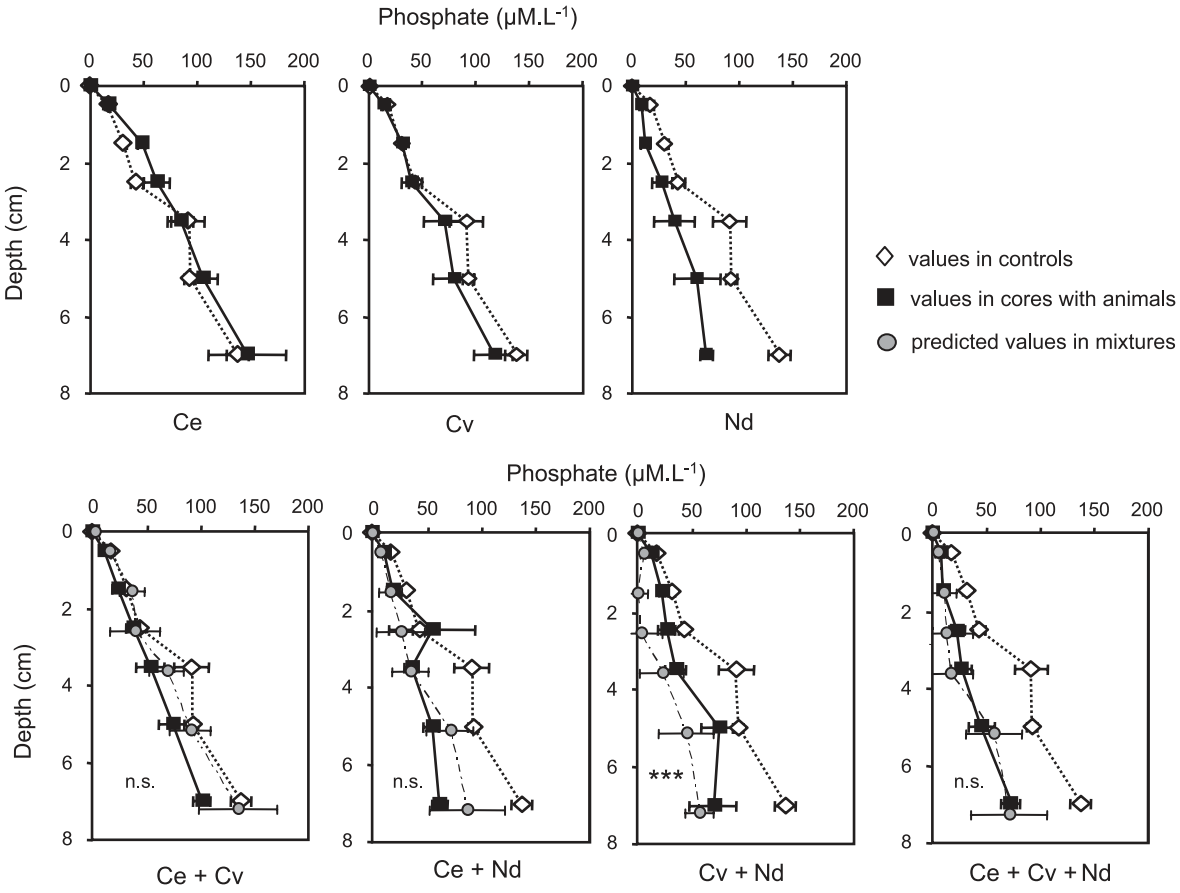

Fig. 6. Vertical profiles of porewater phosphate for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

reductions of ammonium and phosphate concentrations in the sediment were observed with *N. diversicolor* and all treatments containing this species (Nd-, Ce+Nd-, Cv+Nd-, and Ce+Cv+Nd-treatments) produced a similar effect on ammonium and phosphate concentrations in porewater (Tukey HSD tests, p>0.3).

Nitrate concentrations in porewater presented a peak in the top first cm of the sediment in treatments without *N. diversicolor* (Fig. 7). A significant difference in concentration of porewater nitrate was measured among the treatments (one-way ANOVA, p<0.001). *C. edule* had low effect on this solute whereas *C. volutator* significantly increased the concentration of dissolved nitrate in the top 2 cm of the sediment compared to the control (Tukey HSD tests, p<0.001). In contrast, the Nd-treatment 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 (Tukey HSD tests, p<0.001). The Ce+Cv-treatment presented a peak of nitrate at 0.5-cm depth similar (≈30 µM/l) to those observed in the Cv-treatment. In contrast, such peak was not observed in other mixture-treatments (with *N. diversicolor*) that presented similar vertical profiles of nitrate concentration in porewater than these observed in the Nd-treatment (Tukey HSD tests, p>0.05).

At the start of the experiment, total organic carbon in the sediment was 0.8–0.9% dry mass. At the end, a significant loss was observed as values

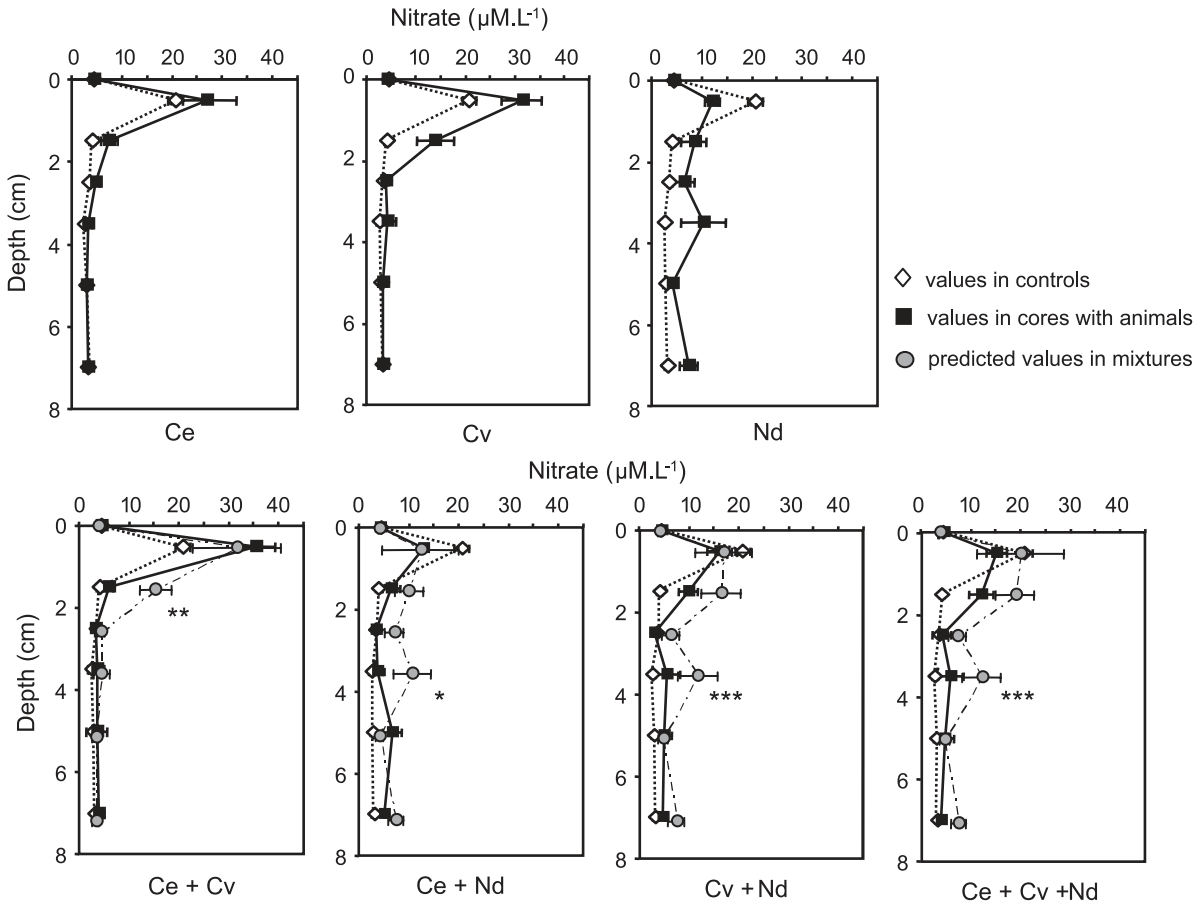

Fig. 7. Vertical profiles of porewater nitrate for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, **p<0.01, *p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

ranged from 0.25% to 0.7% dry mass (Fig. 8). Total organic carbon did not vary significantly with depth (two-way ANOVA, p>0.05) and was different among animal treatments (two-way ANOVA, p<0.01). This significant difference among animal treatments was linked to the significantly lower TOC contents measured in the Cv+Nd and the Ce+Cv+Nd-treatments in comparison with control values, whereas other animal treatments did not affect TOC in the sediment. Although the Ce+Cv+Nd-treatment had a significantly different effect than the N-treatment, no significant difference was measured in TOC profile among the Cv+Nd- and the N-treatment.

Like TOC, total nitrogen decreased from 0.23% to 0.03–0.08% dry mass during the experiment (Fig. 9). TN contents varied significantly with depth (two-way ANOVA, p<0.01) with highest values measured at the sediment surface in most cores. A significant difference was measured among treatments (two-way ANOVA, p<0.01) due to lower TN values measured in the Ce+Cv- and the Cv+Nd-treatments in comparison with the control- and the Ce+Cv+Nd-treatments. However, values in the Ce+Cv- and the Cv+Nd-treatments were in the same range than those measured in one-species treatments.

The total number of bacteria decreased significantly with depth (two-way ANOVA, p<0.001, Fig.

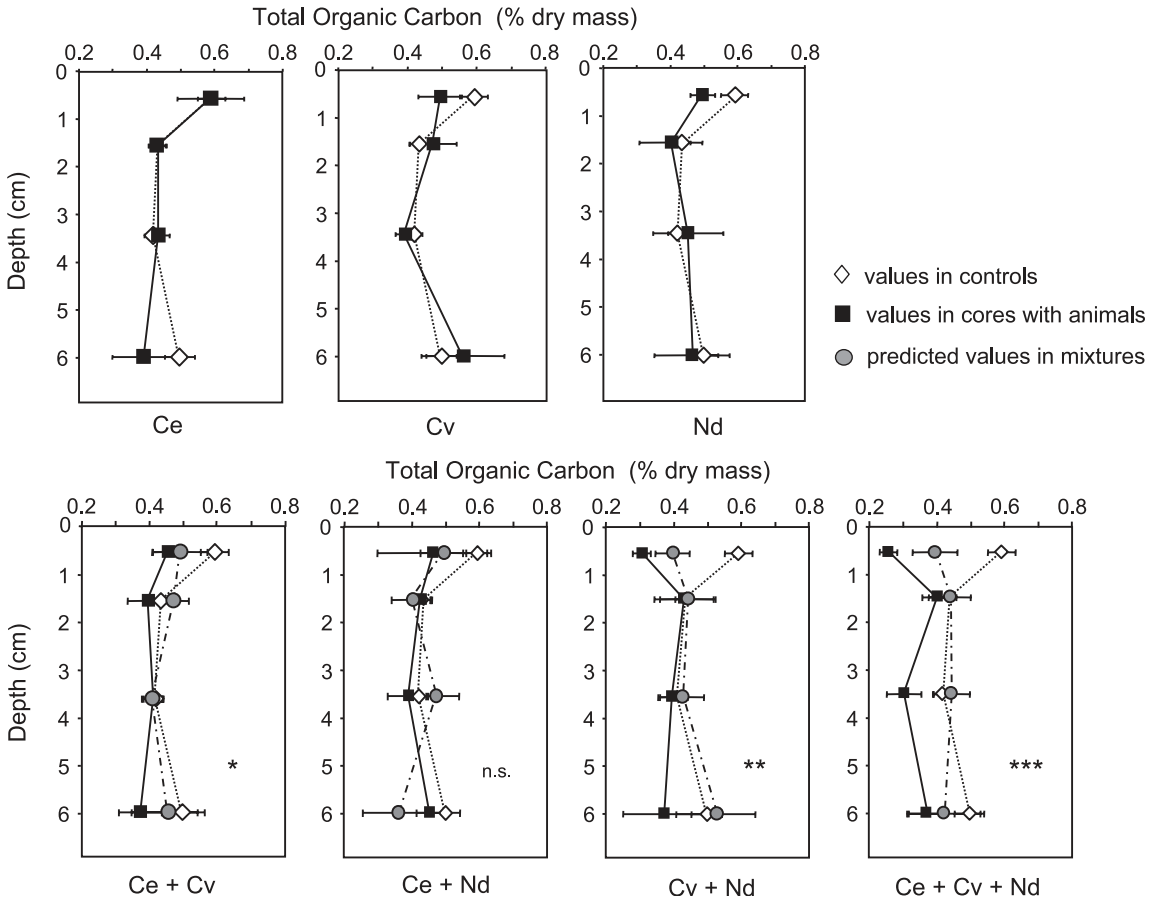

Fig. 8. Vertical profiles of TOC for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (***p<0.001, **p<0.01, *p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

10). A significant difference among treatments was observed (two-way ANOVA, p<0.001). The Ce-treatment did not modify the number of bacteria in the sediment, whereas all other animal treatments tended to increase the bacterial abundances.

The percentage of active bacteria (ratio between bacteria hybridized with EUB and bacteria stained with DAPI) decreased significantly with depth in cores (two-way ANOVA, p<0.001, Fig. 11) and was significantly affected by treatments (two-way ANOVA, p<0.001). The percentages of active bacteria were not significantly modified by Ce- and Ce+Cv-treatments compared to the control (Tukey HSD tests, p>0.8), whereas they were stimulated by all other treatments (Tukey HSD tests, p<0.001). Furthermore, a similar increase in active bacteria was observed in all treatments with *N. diversicolor* (Tukey HSD tests, p>0.25).

The percentage of active sulphate-reducing bacteria was also affected by treatments and decreased with depth (two-way ANOVA, p<0.001, depth and treatment effects, Fig. 12). All animal treatments significantly decreased the percentage of active sulphate reducing bacteria in the sediment compared to the control (Tukey HSD tests, p<0.001). This reduction in sulphate-reducing bacteria was higher in Nd-treatment than in Ce- and Cv-treatments (Tukey HSD test, p<0.01). Mixture treatments with *N. diversicolor* produced a higher decrease in percentages of sul-

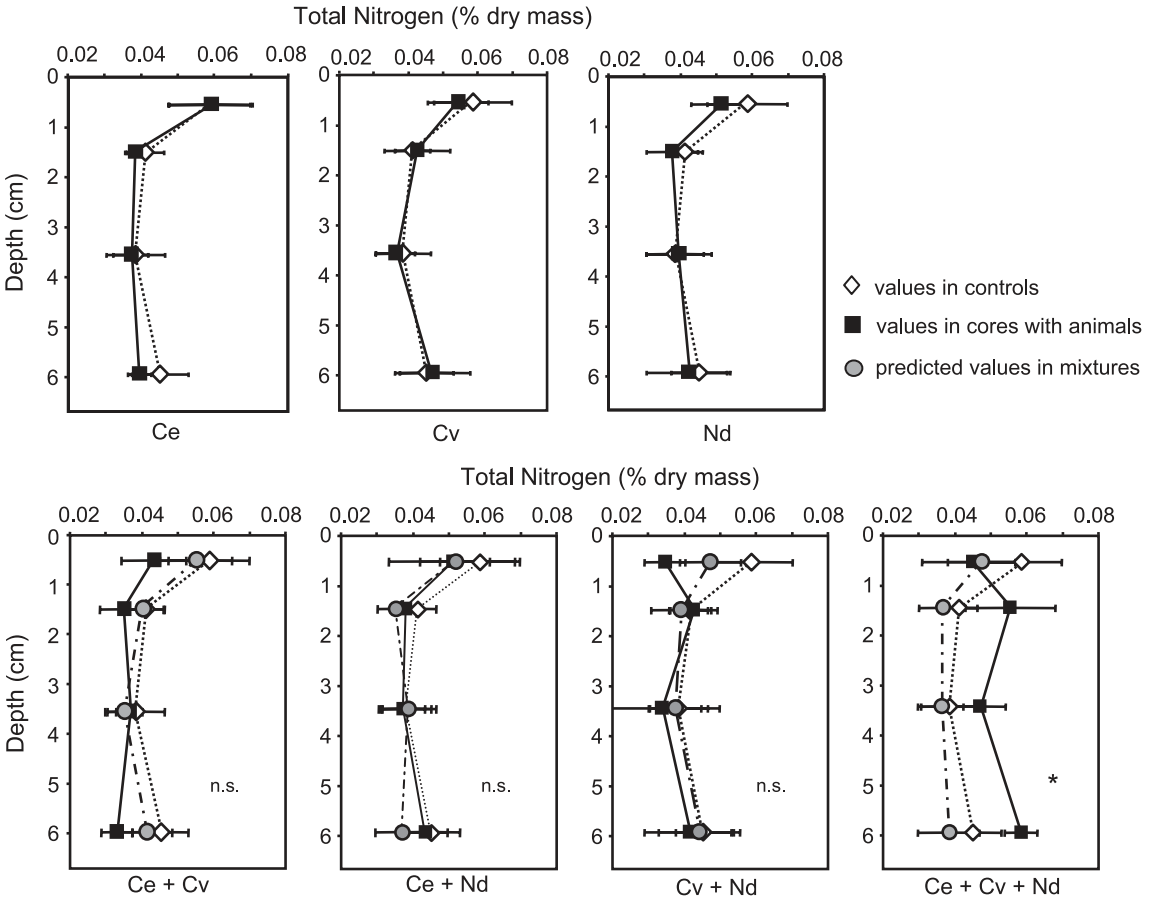

Fig. 9. Vertical profiles of TN for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (*p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

phate-reducing bacteria than one-species treatments (Tukey HSD tests, p<0.001), this decrease being comparable among mixture treatments with *N. diversicolor* (Tukey HSD tests, p>0.1).

3.3. Estimation of the interactions between species

The predicted versus observed coefficients of sediment reworking in animal mixtures are shown on Fig. 2. Whatever the animal treatment, the observed coefficients tended to be lower than the predicted coefficients calculated from individual effects of the species. Significantly lower coefficients of biodiffusion were measured in mixture treatments with *N. diversicolor* in comparison with predictions. The non-local transports of surface sediment measured in the Cv+Nd- and Ce+Cv+Nd -treatments were also significantly lower than those predicted.

Concerning the flux measurements (Figs. 3 and 4), the mixture with Ce and Nd produced similar effects than those predicted. The same result was observed for water exchange and phosphate release in the Ce+Cv-treatment but for other measurements the interaction between Ce and Cv led to lower fluxes than those predicted. The Cv+Nd- and Ce+Cv+Nd-treatments produced significantly lower effects on fluxes than predictions, suggesting a negative interaction among species in these mixture treatments.

The ammonium concentrations in porewater measured in two-species treatments were similar than those

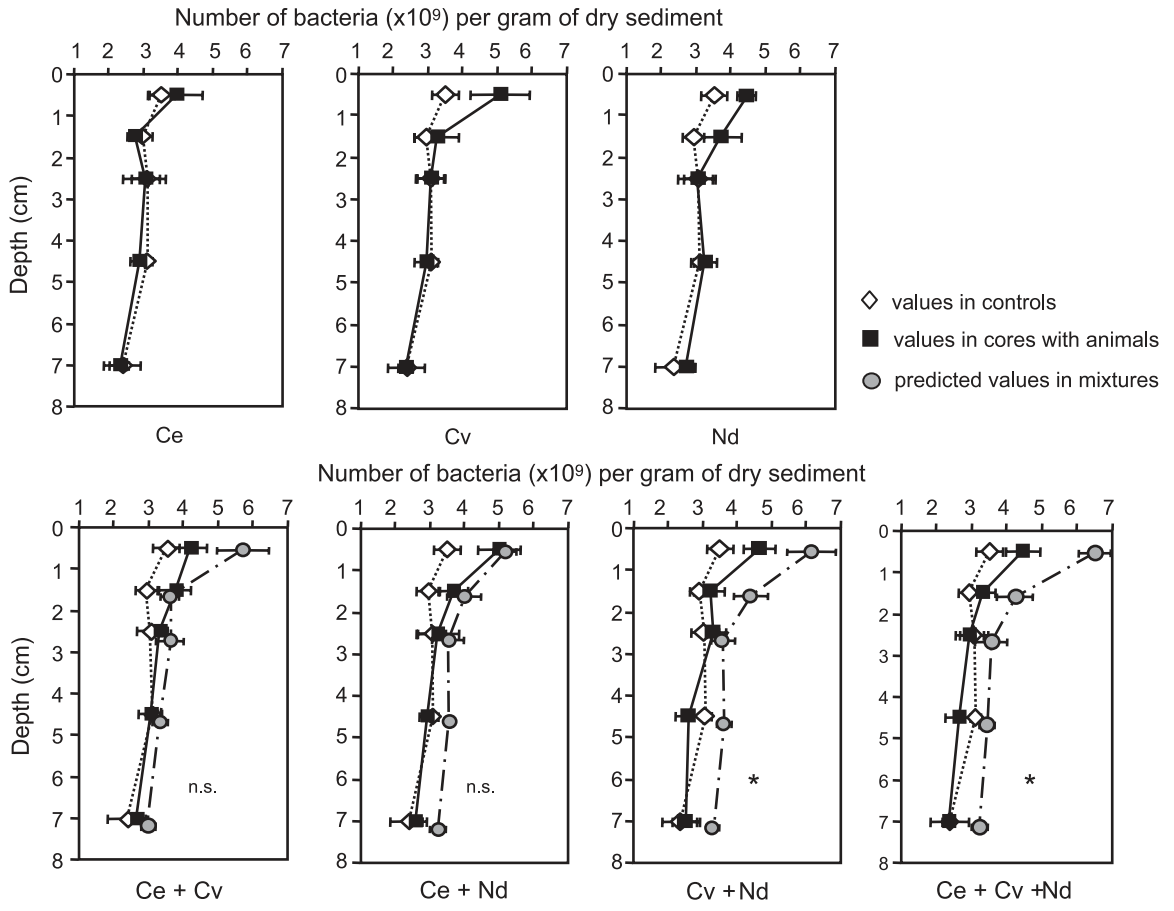

Fig. 10. Vertical profiles of total number of bacteria for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (*p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

predicted (Fig. 5). In contrast, the reduction of ammonium concentration in porewater in the Ce+Cv+Nd-treatment was lower than those predicted. The nitrate concentrations in porewater measured in all mixtures were lower than those calculated from individual effect of each species (Fig. 6). Concerning phosphate measurements in porewater, no significant differences were measured between observed and predicted values except in the Cv+Nd-treatment where diminution of phosphate concentrations in comparison with control cores was lower than prediction (Fig. 7).

All mixture treatments produced significantly lower TOC content in the sediment than those predicted from the additive model except in the Ce+Nd-treatment, indicating a high TOC degradation due to interaction among species (Fig. 8). No differences in total nitrogen content of the sediment were measured between predicted and observed values, except in the Ce+Cv+Nd-treatment (Fig. 9). This latter treatment presented values in the range of the one-species treatments.

For the vertical profiles of bacterial numbers and percentages of active sulphate-reducing bacteria, no significant effects of interactions were measured in the Ce+Cv- and Ce+N-treatments (Figs. 10 and 12). In contrast, the mixtures with Cv and Nd (Cv+Nd and Ce+Cv+Nd) produced lower increases in bacterial abundances and lower decreases of the percentages of

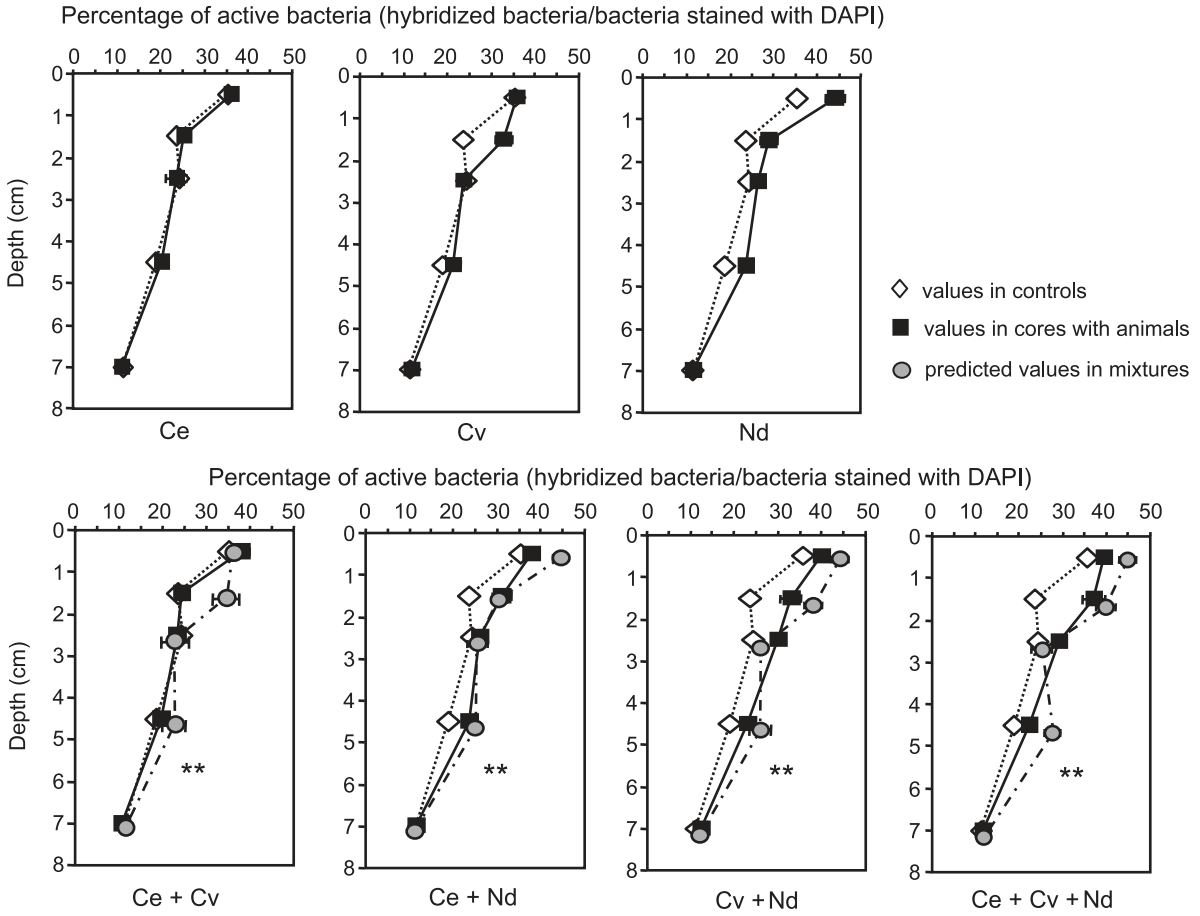

Fig. 11. Vertical profiles of percentage of active bacteria for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (**p<0.01, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

sulphate-reducing bacteria in comparison with control than calculated effects. Concerning the numbers of active bacteria, all mixture treatments indicated negative interactions: measured percentages of active bacteria were lower than those predicted (Fig. 11).

4. Discussion

4.1. Effects of each species

As discussed in a previous paper (Mermillod-Blondin et al., 2004), the results of one-species treatments showed that the three species exhibited different activities of bioturbation (sediment reworking, bioirrigation) in the sediment, leading to specific effects on system functioning. According to several studies in lake (Matisoff et al., 1985), river (Mermillod-Blondin et al., 2002), and marine sediments (Pelegri and Blackburn, 1995; Banta et al., 1999; Christensen et al., 2000; Raffaelli et al., 2003), the effects of each species on sediment processes were strongly influenced by its particular life mode.

Sediment reworking analyses clearly revealed the vertical occupation of the space by the three species. *C. edule* and *C. volutator* were mainly distributed in the top 2–3 cm of the sediment, whereas *N. diversicolor* had a deeper vertical distribution (from 0- to 9-cm

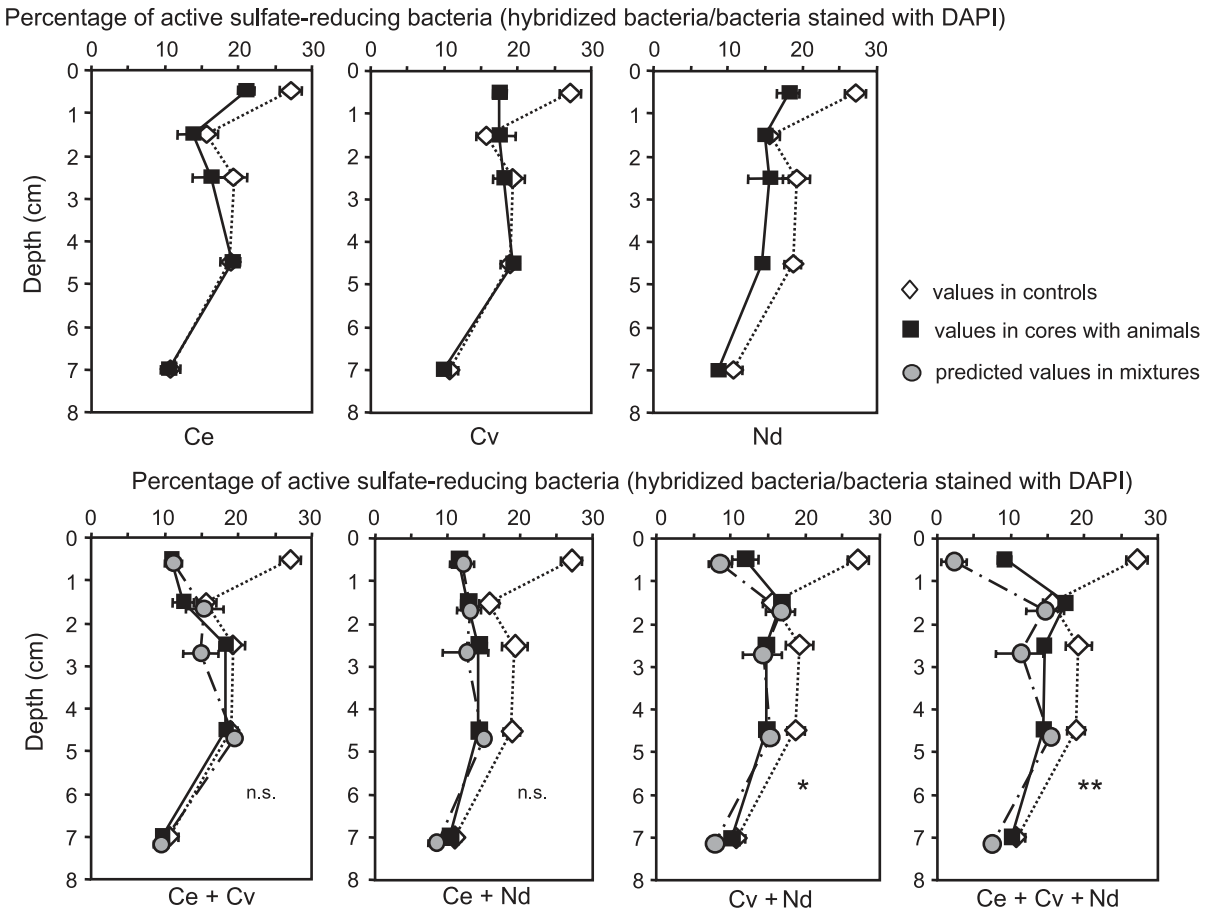

Fig. 12. Vertical profiles of percentage of sulphate-reducing active bacteria for the eight treatments. Profiles from the control cores are indicated on each graph. Error bars are ±1 S.D. of the mean (n=3). Predicted profiles are indicated for the mixture treatments. The results of the statistical comparison between observed and predicted values are indicated on the graphs (**p<0.01, *p<0.05, ns: no significant difference). Abbreviations of animal treatments are as in Fig. 2.

depth). *C. edule* mixed surface particles in the top 2 cm of the sediment. Despite this mixing activity, this species had low effect on O_2 consumption, water exchange between water column and the sediment, microbial characteristics, and release of nutrients from the sediment. As *C. edule* pumps water directly from the overlying water by extending its siphons to the sediment surface, the siphon tissue acts as a barrier for water exchange and so bivalve irrigation does not stimulate water–sediment processes. However, like other species, it reduced the numbers of sulphate reducing bacteria in the sediment. This effect could be attributed to the penetration of O_2 due to its burrowing which reduced the number of active sulphate-reducing bacteria, which are anaerobic, in the microbial community. This assumption is supported by Banta et al. (1999) and Heilskov and Holmer (2001) who showed a reduction of sulphate reduction rates in the upper part of the sediment in the presence of macrofauna. In contrast to *C. edule*, *C. volutator* and *N. diversicolor* produced and ventilated burrows in the sediment which allowed transport of surface particles and solutes into biogenic structures. These two species increased the solute exchange between the water column and the sediment two-fold. These impacts on sediment structure and solute transport stimulated the O_2 consumption, increased the release of nutrients from the sediment, and reduced the concentrations of

ammonium and phosphate in porewater. Both *C. volutator* and *N. diversicolor* stimulated the microbial communities as indicated by higher percentages of active bacteria. However, *N. diversicolor* had a greater impact on porewater chemistry, ammonium sediment release, and active bacteria than *C. volutator*. As *N. diversicolor* burrowed deeper in the sediment than *C. volutator*, it irrigated a greater volume of the sediment, affecting the whole sediment cores. However, as previously showed by Kristensen (1985), *C. volutator* could produce a 2.5 higher release of nitrate from the sediment than *N. diversicolor*. As *C. volutator* acted mainly in the first centimetres of the sediment, it stimulated the oxygenation and the associated nitrification process in this zone. In these conditions, the nitrate produced was rapidly exported to the overlying water column. *N. diversicolor* produced peaks of nitrate in deeper layers of the sediment due to ventilation of burrows which could stimulate the nitrification process as was shown for several burrowing animals (Kristensen et al., 1985; Aller, 1988; Mermillod-Blondin et al., 2004). As opposite to the nitrate produced at 0–1 cm depth due to *C. volutator*'s activity, the nitrate produced at depth by *N. diversicolor* was not transported to the water column (or it was transformed during the transport). Therefore, the three species had different activity patterns (sediment reworking, bioirrigation, burrowing depth), leading to different effects on sediment processes.

4.2. Impact of diversity on biogeochemical processes

Measurements of interactions showed that most mixture treatments produced lower effect than those predicted from the additive model using effects of one-species treatments. For example, bromide exchange and O_2 uptake increased two-fold with the Cv- and Nd-treatments in comparison with the control. However, mixtures with these two species (Cv+Nd- and Ce+Cv+Nd-treatments) produced a lower increase (two-fold increase) of these processes in comparison with predictions by the additive model (4-fold increase). This type of negative interaction measured between species on ecosystem processes might result from an overlap of bioturbation activities among the three species which lived and foraged in the same habitat (water–sediment interface). In these conditions, our results suggest that, in presence of several species that use and modify the same sediment space, impact of invertebrates on ecosystem processes was essentially due to the most efficient bioturbator of the community (*N. diversicolor*). The luminophore analyses showed that all treatments with *N. diversicolor* exhibited comparable sediment reworking rates characterized by a gallery-diffusion process. In comparison, treatments without *N. diversicolor* (Ce-, Cv-, and Ce+Cv-treatments) gave lower sediment reworking activities. In all treatments with *N. diversicolor*, the same modifications of porewater chemistry were measured in the sediment: a reduction of ammonium and phosphate concentrations and a production of nitrate at depth. Nutrient releases from the sediment (that can explain the diminution of nutrient concentrations in porewater by pore solute flushing, Banta et al., 1999) were comparable in all treatments with *N. diversicolor* whatever the species associated with the worm. Treatments with *N. diversicolor* also produced the highest stimulation of active bacteria and decrease of active sulphate-reducing bacteria, indicating a predominant effect of *N. diversicolor* on microbial characteristics. In these conditions, the strong effects of *N. diversicolor* on sediment reworking, water exchanges, O_2 uptake, nutrient fluxes, porewater chemistry, and bacterial characteristics hid the impacts of the other species. For example, *C. volutator* in single treatment stimulated the release of nitrate from the sediment, whereas this release was not additive in the presence of *N. diversicolor*. Analyses of porewater chemistry showed that the presence of *N. diversicolor* suppressed the combined production of nitrate at the 0–1-cm depth layer observed in the Cv-treatment. In such conditions, nitrate was poorly exported to the overlying water in comparison with treatments producing a peak of nitrate in the upper cm of the sediment (Cv- and Ce+Cv-treatments). Therefore, the modification of porewater chemistry by *N. diversicolor* had an impact on all nutrient fluxes at the water–sediment interface. As the area of the sediment bioturbated (bioirrigated) by *N. diversicolor* comprised the shallow zones burrowed by *C. edule* and *C. volutator*, the impact of these latter species in the top 2 cm of the sediment was hidden by the strong impact of *N. diversicolor* on the whole sediment core. For example, in the Cv+Nd-treatment, ventilation of burrows by *N. diversicolor* also modified the layer of the sediment inhabited by

C. volutator. In consequence, the area burrowed by *C. volutator* was influenced by the bioirrigation of *N. diversicolor*, leading to lower sediment–water fluxes than the additive model would predict. Thus, results from our experiments agree with those of Emmerson et al. (2001) and Raffaelli et al. (2003), who noted that sediment–water processes were more sensible to species identity than species diversity. In other words, the presence of a particular species in the ecosystem may be more important on the biogeochemical processes than the species diversity. For example, Vopel et al. (2003) showed that an ubiquous brittle star (*Amphiura filiformis*) in the north sea can account for 80% of O_2 uptake in soft sediment, suggesting that this brittle star is a key species at the water–sediment interface.

According to the O_2 uptake rates measured during our experiments, mixtures should not produce higher TOC consumption than those predicted from the additive model. However, this was observed in most mixture treatments, the Ce+Cv+Nd-treatment producing lower TOC than all one-species treatments. The TN measurements also indicate significant interactions between species that were not clearly linked to nitrogen fluxes at the water–sediment interface. For example, the Ce+Cv+Nd-treatment significantly increased the TN content in the sediment, whereas the Cv+Nd-treatment decreased the nitrogen content despite similar ammonium fluxes and O_2 uptake in both treatments. The lack of consistence between TN and TOC measurements and flux measurements (oxygen uptake and nitrogen release) may result from the complex influence of animal burrowing on the particulate organic matter at a fine scale (Kristensen et al., 1985; De Vaugelas and Buscail, 1990). 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 stock of C and N in different locations of the sediment depending on distance from water–sediment interface and from burrows. In consequence, analysis of small samples was not adequate to measure the wider influence of animals in the sediment. Furthermore, the strong reduction of TN in all cores (more than 74%) reduced the ability to detect treatment differences. Finally, it seems that analyses of TOC and TN need to be more precise (ex: 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 treatments on TOC and TN in the sediment.

The present study showed that the role of species and species diversity on fluxes could benefit from the assessment of porewater analysis to understand how each species and combinations of species affect water–sediment fluxes. For example, the results of nitrate release from the sediment could not be interpreted without assessing the nitrification zone in the sediment. In the same way, analysis of sediment reworking is essential to describe the activities and the space occupied by invertebrates in the sediment, determining their impact at the water–sediment interface (François et al., 1997, 1999; Mermillod-Blondin et al., 2004). Thus, determination of the impact of invertebrate diversity on water–sediment processes needs a description of most activities played by invertebrates in the sediment.

In shallow water area dominated by the three species studied in the present work, *N. diversicolor* is likely to have significant impact on biogeochemical processes in the sediment by its extensive activity. This species has a strong impact on fluxes and sediment characteristics (chemical and physical) which is suggested to hide the particular role of the other species in mixture treatments. *N. diversicolor* could be characterized as a keystone species (as defined by Mooney et al., 1995) which presents particular traits (burrowing depth, bio-irrigation behaviour, sediment reworking activity) that have predominant impacts on sedimentary processes. According to several studies in both terrestrial and aquatic systems (Hooper and Vitousek, 1997; Loreau et al., 2001; Fridley, 2002; Emmerson et al., 2001; Jonsson and Malmqvist, 2003b), our findings suppose that the functional traits of the individual species in a community are more important than species richness in order to determinate the relationship between biodiversity and ecosystem processes.

Although our results demonstrated the dominance of *N. diversicolor* on ecosystem functioning, our study used a simplified experimental system with a given density of each species in treatments. For example, the impact of density (biomass) of each species and assemblages of species on ecosystem functioning has not been tested because our additive

model supposed that animal density did not strongly affect the impact of each species. The similar results observed in all treatments with *N. diversicolor* support our assumption because this species influenced the ecosystem processes in the same way whatever the presence of another species. However, further investigations using variable densities of each species in assemblages should be developed to analyze the possible density-dependent impact on each ecosystem process.

Future works should also include more functional groups of species in order to determine the functional traits of species which influence biogeochemical processes in the sediment. For example, in shallow waters, the importance of predation for the community structure has been widely demonstrated (Paine, 1980; Reise, 1985; Menge et al., 1994). However, the functional impact of a strict predator on biogeochemical processes (by acting on the behaviour of bioturbator preys for example) has to be tested in order to determine the role of trophic interactions in the biodiversity–ecosystem processes relationship. Studies could be expanded to include more functional groups of infauna (predators, upward and downward conveyors) which can influence in a specific way the functioning of marine sediments. Biles et al. (2003) showed the significant influence of physical conditions (hydrodynamics) on the role of invertebrate diversity on nutrient fluxes at the water–sediment interface. In two shallow bays of the Sweden west coast, Sundbäck et al. (2003) demonstrated that the interactions among sediment characteristics, algal, and zoobenthic communities strongly influence the nutrient and O_2 fluxes at the water–sediment interface. Thus, there is a need to develop our experiments in a wider perspective to evaluate the importance of invertebrate diversity on ecosystem functioning.

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