

# Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation

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**ABSTRACT:** The importance of different functional traits of macrobenthos in benthic processes of the Southern Bight of the North Sea was investigated to estimate the effects of density declines and species loss on benthic ecosystem functioning. Two laboratory experiments were performed: before (winter, temperature = 10°C) and after (summer, temperature = 18°C) sedimentation of the spring phytoplankton bloom. Single species treatments of key species (*Abra alba*, *Lanice conchilega* and *Nephtys* sp.) with different functional traits were added to microcosms at 3 density levels (natural, lower, lowest) to account for possible density declines. Sediment–water exchanges of oxygen and nutrients, denitrification and bioturbation were measured. In absence of fauna, benthic mineralisation in the summer experiment was 2.0 times higher than in winter. Fauna stimulated microbial respiration more in summer (up to 100 % in *L. conchilega* treatments) than in winter (negligible fauna effect). As chlorophyll *a* concentrations were similar in both seasons, the stronger fluxes in summer must be explained by a higher macrobenthic activity owing to the elevated temperature and better condition of the animals. Stimulation of mineralisation by the 3 species in the microcosms was different, and behaviour-related. Owing to its irrigation activity, the tube dweller *L. conchilega* had more pronounced influences on benthic respiration, nutrient release and denitrification than did the biodiffusers, *A. alba* and *Nephtys* sp. *A. alba* appeared to be a more effective bioturbator than *Nephtys* sp. Processes such as benthic respiration, nutrient fluxes, denitrification and bioturbation seem to be related to animal densities and therefore decreases in densities can possibly have implications for ecosystem functioning.

**KEY WORDS:** Ecosystem functioning · Macrobenthos · Functional traits · Density · Temporal variability · *Lanice conchilega* · *Abra alba* · *Nephtys* sp.

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## INTRODUCTION

Macrofauna-induced particle mixing (bioturbation) and solute transfer (bio-irrigation) contribute extensively to ecosystem functioning in areas where physical disturbance is low (Kristensen & Kostka 2005, Meysman et al. 2006). In search for food, macrobenthic organisms actively rework and irrigate the sediment. Oxygen and organic matter are transported deeper

into the sediment and transfer of excretion products to the water column is enhanced. Furthermore, these organisms stimulate mineralisation (Yingst & Rhoads 1980, Mermillod-Blondin et al. 2004), increase nutrient turnover and therefore renew the pool for primary production (Blackburn 1988).

Due to disturbance of the seabed, be it natural or anthropogenic, e.g. trawling (Bergman & Hup 1992), dredging (Rhoads et al. 1978) and hypoxia (Van Colen

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et al. 2008) resulting from eutrophication or global warming, macrobenthos densities and community structure can change (Pearson & Rosenberg 1978, Thrush et al. 2006). Removal of ecosystem engineers (organisms that influence the resources of other biota via changes in the environment; see Jones et al. 1994) such as bioturbators and bio-irrigators could induce large changes in the structure of the habitat, with cascading effects on local biodiversity (Coleman & Williams 2002) and ecosystem functioning, such as benthic mineralisation processes (Olsford et al. 2008, Rossi et al. 2008). Thus, it is of critical importance to study the role of species densities in ecosystem functioning (Marinelli & Williams 2003).

Much progress has been made in describing the role of different functional macrobenthic traits in marine ecosystem functioning (Mermillod-Blondin et al. 2004, Michaud et al. 2005, 2006, Norling et al. 2007, Rossi et al. 2008). Several species have been recognized as ecosystem engineers (Vopel et al. 2003, Maire et al. 2007, Volkenborn et al. 2007), because of their mediating effects on biogeochemistry. There is, however, still a need for identifying the effects of other possible ecosystem engineering species on biogeochemical cycling. These case studies are necessary to provide basic material that can be integrated into the broad ecosystem engineering concept (Wright & Jones 2006), which is important for conservation concerns. Some work has focused on the effects of macrobenthic density (e.g. Marinelli & Williams 2003, Duport et al. 2006) on sediment–water exchange and sediment reworking, but few researchers have studied the effect of temporal variability (Ouellette et al. 2004, Maire et al. 2007).

The goal of this study was to extend the experimental work of the last decades by integrating different factors (functional traits, density and temporal variability) influencing ecosystem functioning. The study site, situated in the Belgian Western Coastal Banks, has been put forward as an area that deserves marine protection. The area is of high ecological value and is dynamic in terms of sediment metabolism (Van Hoey et al. 2004, Franco et al. 2007, 2008). Sedimentation of the spring phytoplankton bloom strongly influences biogeochemical gradients in the sediment and bioturbation is thought to be an important factor in structuring these patterns (Vanaverbeke et al. 2008).

To verify this hypothesis, the influence of 3 abundant species with functionally different traits on benthic ecosystem processes was assessed. *Abra alba*, a bivalve, reworks the upper layer of the sediment at random and is therefore called a biodiffuser (Gerino et al. 2003, Maire et al. 2007). *Lanice conchilega*, a tube building polychaete, acts as a piston pumper (Forster & Graf 1995) and can occur in high density patches,

referred to as reefs (Rabaut et al. 2009). These reefs host a wealth of associated species (e.g. Callaway 2006, Rabaut et al. 2007); therefore, *L. conchilega* is considered an ecosystem engineer of high functional value and in need for conservation (Godet et al. 2008). Both *A. alba* and *L. conchilega* are suspension–deposit feeding organisms. *Nephtys* spp. are predatory polychaetes that act as biodiffusers by creating transient burrows (Hartmann-Schröder 1996).

In the present work, single species treatments of each of the 3 species were incubated in 3 densities: (1) their natural density, (2) a lower and a (3) lowest density, to investigate the effect of possible density declines on benthic ecosystem functioning, as measured by sediment–water exchange rates of oxygen, dissolved inorganic nitrogen ( $\text{DIN} = \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$ ) and dinitrogen gas ( $\text{N}_2$ ), (i.e. denitrification) and bioturbation. The influence of temporal variability was assessed by setting up 2 experiments, one performed in winter before the phytoplankton bloom deposition when macrobenthos was naturally starved, and one in summer when macrobenthos had been feeding on the large amount of organic matter (OM) deposited during and after the spring phytoplankton bloom. The null hypotheses of this study were that (1) the functional traits of the macrobenthic species, (2) density of the species and (3) temporal variability would not affect biogeochemical fluxes or sediment reworking.

## MATERIALS AND METHODS

**Field sampling.** Sediment from the fine sandy coastal Stn 115bis (51° 09.2' N, 02° 37.2' E; 13 m depth) in the Western Coastal Banks area was collected in January and July 2007 with a Van Veen grab from the RV 'Zeeleeuw'. Characteristics of the benthic communities at Stn 115bis are given in Vanaverbeke et al. (2008) and van Oevelen et al. (2009). Sediment was defaunated by sieving over a 1 mm mesh, followed by freezing and thawing (Schratzberger et al. 2004), and stored frozen (–20°C) for 6 wk until the start of the experiment. This defaunation method eliminates macro- and meiofauna but does not eliminate bacterial populations (Stocum & Plante 2006). The median grain size of the sediment was 185 µm and consisted of 14 % silt (van Oevelen et al. 2009). Pigment concentrations in water and sediment were determined in February and September. Water samples were collected with 10 l Niskin bottles at 3 m below the water surface and 1 m above the sea floor. Three replicate samples of 500 ml each were filtered onto GF/F glass microfiber filters. Sediment was sampled deploying a Reineck box corer (180 cm<sup>2</sup>). From 3 box corers, a Perspex core (inside diameter [i.d.] 3.6 cm) was sliced in 1 cm hori-



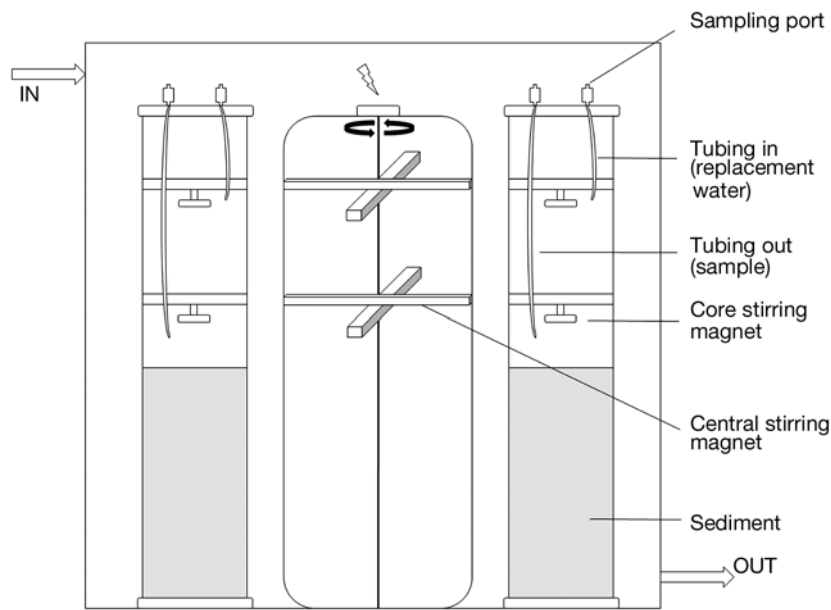


Fig. 1. Experimental set-up. Plexiglas cores submerged in a tank with continuous flowthrough of seawater from the Oosterschelde. Cores are shown as during flux measurements (capped)

ment used for the incubations (lids, Teflon tubings, Luer stopcocks) was submerged in the incubation tank 48 h before measurements were taken to avoid introduction of new surfaces for  $O_2$ ,  $N_2$  and argon (Ar) adsorption. This is pivotal for the correct determination of  $N_2$  production (Kana et al. 1994). Each core was sealed with an airtight lid and incubated in darkness for 36 h (March) and 8 h (September), depending on the change in overlying water dissolved oxygen concentration, which never decreased below 50% air saturation.

Sample collection was performed as described in Na et al. (2008). The concentrations of  $N_2$  and Ar were determined by membrane-inlet mass spectrometry (MIMS) with an Omnistar QMS 200 quadrupole mass spectrometer (Baltzers Instruments).  $N_2$  and  $O_2$  concentrations were normalised to those of Ar (Kana et al. 1994) and the  $N_2$  production and  $O_2$  consumption calculated from the linear increase and decrease, respectively in Ar-normalised  $N_2$  and  $O_2$  concentration and corrected for refill water (Na et al. 2008). DIN samples were filtered through GF/F filters (Whatman), frozen immediately and analysed using automated colourimetric techniques. Net DIN fluxes were calculated from the difference between initial and final samples compensating for dilution by refill water. Vertical oxygen profiles (5 per core) were measured in September with oxygen microsensors (type ox25, Unisense) in vertical increments of 250  $\mu\text{m}$ .

**Bioturbation measurements.** To estimate bioturbation activity of *Abra alba* and *Nephtys* sp., luminophores (Environmental Tracing Systems) were used as tracers for

sediment transport. White and pink luminophores of the same size as the sediment median grain size (180  $\mu\text{m}$ ) were placed as frozen mud cakes (1.7% luminophores, 5 mm thickness) on the surface (white) and within the sediment column (pink, 4 cm depth) to investigate downward and upward transport, respectively (Gerino et al. 1998). After 14 d, water was carefully siphoned off and cores were stored at  $-20^\circ\text{C}$ . Cores were subsequently sliced in 5 mm sections down to 6 cm depth, then 1 cm sections to 10 cm depth. Image analysis of UV-illuminated pictures (Canon 350D digital SLR camera) of oven dried and carefully homogenised sediment sections resulted in vertical luminophore profiles. Bioturbation rates were calculated by fitting a non-local transport model to these luminophore profiles. We used a non-local instead of the classical biodiffusion model, because the assumptions of the latter are not fulfilled in short-term experiments (Meysman et al. 2008), especially when bioturbation rates are low. We

used the Continuous Time Random Walk model (Meysman et al. 2008), with a Poisson stepping process and a Gaussian step-length distribution. A single value for the non-local biodiffusion coefficient as a measure for mixing intensity,  $D_b^{NL}$  in  $\text{cm}^2 \text{yr}^{-1}$ , is calculated from the fitted parameters  $\sigma$  (characteristic step length) and  $\tau$  (average waiting time) as:

$$D_b^{NL} = \frac{\sigma^2}{2\tau} \quad (1)$$

It was not relevant to measure bioturbation activity of *Lanice conchilega* since this species resides in its semi-permanent tube and does not rework the sediment column.

**Estimation of faunal respiration.** Biomasses were used to estimate macrofaunal respiration. Wet weights of *Abra alba* and *Nephtys* sp. were converted to ash-free dry weight (AFDW) using seasonally dependent conversion factors (Sistermanns et al. 2004). AFDW of *Lanice conchilega* was estimated from mean tube diameter (Van Hoey 2006). For all species, 50% of this AFDW was considered to be carbon. Respiration rate was calculated by means of the Mahaut et al. (1995) formula for shallow water macrobenthos:

$$R = 0.0174 W^{0.844} \quad (2)$$

where  $R$  is respiration ( $\text{mg C d}^{-1}$ ) and  $W$  is mean individual AFDW ( $\text{mg C}$ ), valid for the temperature range of 15 to  $20^\circ\text{C}$ . Respiration was then corrected for temperature assuming a  $Q_{10}$  of 2 and converted to oxygen consumption assuming a respiratory quotient of 0.85.

**Mass budget calculation.** The sediment–water exchange fluxes of  $O_2$ ,  $NO_x$  and  $NH_x$  were used to quantify denitrification, nitrification, total carbon and nitrogen mineralization. This was done by constructing an integrated mass budget of oxygen, nitrate and ammonium in the sediment as a function of the source and sink processes (Soetaert et al. 2001). Oxygen is either directly consumed to oxidise organic carbon (oxic mineralization [OxicMin]), or indirectly through the re-oxidation of reduced substances formed by anoxic mineralization (AnoxicMin). To re-oxidise all reduced substances formed upon anoxic mineralization, one mole of oxygen is consumed for each mole of carbon originally mineralised. Part of the reduced substances can remain buried in the sediment (pSolidDepo) and is therefore not re-oxidised. It is assumed that the (molar) O:C ratio of organic matter equals 1. In addition, 2 moles of oxygen are required to oxidise ammonium to nitrate (Nitrification). Ammonium is the product of the mineralisation of organic nitrogen (Nmineralisation), but it is consumed by nitrification. Nitrification produces nitrate, but Denitrification consumes it; 0.8 moles of  $NO_3$  are consumed for 1 mole of carbon denitrified. Oxygen, nitrate and ammonium are exchanged through the sediment–water interface ( $O_2$ inFlux,  $NO_x$ inFlux,  $NH_x$ inFlux).

These mass balances are summarised below.

$$\begin{aligned} \frac{dO_2}{dt} &= O_2\text{inFlux} - \text{OxicMin} - \text{AnoxicMin} \times \\ &\quad (1 - \text{pSolidDepo}) - \text{Nitrification} \times 2 \\ \frac{dNH_x}{dt} &= NH_x\text{inFlux} + \text{Nmineralisation} - \text{Nitrification} \\ \frac{dNO_x}{dt} &= NH_x\text{inFlux} + \text{Nitrification} - \\ &\quad \text{Denitrification} \times 0.8 \end{aligned} \quad (3)$$

The exchanges across the sediment–water interface ( $O_2$ inFlux,  $NH_x$ inFlux,  $NO_x$ inFlux) were estimated during the incubation experiments, while the rate of change of oxygen ( $dO_2/dt$ ), nitrate ( $dNO_x/dt$ ) and ammonium ( $dNH_x/dt$ ) fluxes was assumed to be zero (geochemical steady state reached 1 wk after introducing organisms). With 5 remaining unknowns (OxicMin, AnoxicMin, Nmineralization, Nitrification, Denitrification) and only 3 equations, the mass balance model has no unique solution. Therefore, we make the assumption that the burial of anoxic substances can be ignored ( $\text{pSolidDepo} = 0$ ). This allows combining the oxic and anoxic mineralization into one quantity (oxicAnoxicMin). The extra equation to balance the model then imposes a relationship between nitrogen and carbon mineralization, using the average N\_C ratio as measured in the sediment. This ratio equalled 0.1959 ( $\text{mol N mol C}^{-1}$ ). The mass balances then become:

$$\begin{aligned} 0 &= O_2\text{inFlux} - \text{OxicAnoxicMin} - \text{Nitrification} \times 2 \\ 0 &= NH_x\text{inFlux} + \text{Nmineralisation} - \text{Nitrification} \end{aligned} \quad (4)$$

$$0 = NO_x\text{inFlux} + \text{Nitrification} - \text{Denitrification} \times 0.8$$

where,

$$\text{Nmineralisation} = (\text{OxicAnoxicMin} + \text{Denitrification}) \times \text{N:C ratio}$$

These 3 equations can be solved for the 2 unmeasured quantities (OxicAnoxicMin, Nitrification) using least squares techniques. However, when measured denitrification rates were introduced in the mass budget, the model did not fit well with the other introduced quantities. As the measurement of denitrification was considered to be the least accurate, we chose to run the mass budget considering denitrification as an extra unknown to be fitted, i.e. imposing only  $O_2$ inFlux,  $NH_x$ inFlux and  $NO_x$ inFlux as input. With 3 equations and 3 unknowns this makes the model evenly determined. The modelled denitrification can then be compared with our measured rates *a posteriori*. The mass balance modelling was done using package `limSolve` (Soetaert et al. 2008) available in the open source software R (R Development Core Team 2009).

We chose to make measurements of several aspects of the N cycle rather than analyse a large number of replicates of fewer variables using a variance based statistical approach. The latter strategy allows for detection of significant differences in the measured variables (Benedetti-Cecchi 2004), but we aimed to obtain a more holistic view by performing measurements of different N-cycle related processes covarying with ammonium fluxes (nitrate fluxes, oxygen fluxes,  $N_2$  fluxes) in combination with modelling of individual flux terms subjected to overall mass balance constraints. This allowed us to (1) assess the robustness of single measurements and (2) understand why patterns were observed.

**Statistical analyses.** Student's *t*-tests were performed with Statistica 6.0 software to test for differences in pigment concentrations between winter and summer. Homogeneity of variances was verified with a Levene's test. Data were log transformed when the assumptions were not met. Relations between densities and measured fluxes, stimulation of sediment community oxygen consumption (SCOC),  $D_b^{NL}$  and  $D_b^{NL}$  standardised for biomass were tested with linear regression analysis. Assumptions for the use of linear regressions were verified graphically. Normality of residuals was confirmed numerically with a Shapiro-Wilks test. When the assumption of linearity was not observed and it was biologically relevant to do so, a lack of fit test was performed to check whether a quadratic function described the data better than a linear one. Additional analyses of covariance (ANCOVA)

were conducted to compare slopes of regressions. The level for statistical significance was set at 0.05 and only significant relations are shown in tables. Results are shown as means  $\pm$  SE.

## RESULTS

### *In situ* sediment characteristics

Chl *a* concentrations in the water column were low in February (winter) ( $1.05 \pm 0.04 \mu\text{g l}^{-1}$  and  $1.36 \pm 0.23 \mu\text{g l}^{-1}$  in surface and bottom water, respectively). This applies to the sediment as well: in the top 5 cm  $26.99 \pm 4.77 \text{ mg chl } a \text{ m}^{-2}$  was found in February. In late August (summer), water chl *a* concentrations were higher ( $7.28 \pm 0.87 \mu\text{g l}^{-1}$  and  $3.96 \pm 1.04 \mu\text{g l}^{-1}$  in surface and bottom water respectively) (Student's *t*-test,  $t = -12.76$ ,  $p = 0.0002$  and  $t = -3.66$ ,  $p = 0.02$ , respectively). Sediment concentrations were  $33.71 \pm 4.6 \text{ mg chl } a \text{ m}^{-2}$  in the top 5 cm sediment and, thus, comparable with winter values) (Student's *t*-test,  $p = 0.22$ ).

### Bioturbation

The surface of control cores was smooth, with a brown–gray zone reaching 0.5 cm into the sediment. Below this oxic–suboxic zone, the sediment was black. In both winter and summer, all surfaces of *Abra alba* treatments were bumpy, with a grayish zone extending to 2 cm depth. Actual and deserted feeding pits (Maire et al. 2007) were visible along the core walls. The depth of the grayish zone in *Lanice conchilega* cores did not differ from control cores. During the whole experiment *L. conchilega* was very active, which was evident by the length (up to 10 cm) of the reconstructed tubes above the sediment surface and the number of newly built fringe rims. Much surficial sand had been replaced for the construction of these fringes. Where polychaetes had constructed their U-shaped tubes with one leg of the U against the core wall, a grayish zone of 5 mm was visible along the tubes reaching down to 10 cm depth. The grayish zone in *Nephtys* sp. cores was similarly shallow, but the remains of burrows, which were very narrow conical structures, were abundant along the core wall.

At the end of the winter experiment, *Abra alba* specimens were recovered in the first 5 cm of the sediment while the individuals remained in the upper 2.5 cm in summer. *Nephtys* sp. were found at 3.5 and 4 cm depths in winter and summer, respectively.

The bioturbation rate  $D_b^{NL}$  in control cores was very low ( $0.22 \pm 0.05 \text{ cm}^2 \text{ yr}^{-1}$ ), indicating that the observed luminophore displacements in animal cores resulted

from bioturbation. In all treatments, deep tracers were recovered at the depth of introduction, indicating that particle mixing was restricted to the top few centimetres. In winter, 2% of the luminophores administered to the surface were recovered below 0.5 cm in control cores, 15 to 37% were recovered in *Abra alba* sediments (from lowest to natural density) and 4 to 22% (both replicates of low density) were recovered in *Nephtys* sp. sediments. In summer, 1 to 5% luminophores were recovered below 0.5 cm in control cores, 13 to 42% were recovered in *A. alba* (from lowest to natural density) cores and 5 to 10% were recovered in *Nephtys* sp. cores (from natural to low density).

Bioturbation activity increased significantly (Fig. 2, Table 2) with *Abra alba* density in winter and not significantly (but nearly so) in summer ( $p = 0.051$ ) ( $D_b^{NL}$  range from 0.96 to  $4.47 \text{ cm}^2 \text{ yr}^{-1}$ ) but not with density of *Nephtys* sp. ( $D_b^{NL}$  range from 0.29 to  $1.22 \text{ cm}^2 \text{ yr}^{-1}$ ). Bioturbation rate of *A. alba*, standardised for grams (g) AFDW, showed no significant density dependence, implying that individual  $D_b^{NL}$  was identical among density treatments. *Nephtys* sp. bioturbation rate standardised for biomass tended to diminish with increasing densities in both winter and summer, but not significantly, however.

### Oxygen penetration depth and consumption

In summer, oxygen was generally depleted at 3 mm depth, except in the *Lanice conchilega* cores (Fig. 3). While oxygen penetration in between tubes ( $4.4 \pm 0.3 \text{ mm}$ ) was similar to observations in control cores ( $3.2 \pm 0.2 \text{ mm}$ ), there was a deeper penetration near the tubes ( $6.3 \pm 1.6 \text{ mm}$ ). This oxygen penetration depth tended to increase with *L. conchilega* densities, but this relation was not significant.

Control SCOC in the summer experiment was 2.0 times higher than in the winter experiment. In winter, SCOC increased significantly with densities of *Abra alba* and *Lanice conchilega* (Table 2), but not with *Nephtys* sp. densities. SCOC depended more on *L. conchilega* densities than on *A. alba* densities (ANCOVA,  $p < 0.05$ ). Summer SCOC was significantly related only to *L. conchilega* densities, but more strongly than in winter (ANCOVA,  $p < 0.001$ ). Linear regression analysis in function of biomass yielded very similar results as the results in function of density (Table 3, Fig. 4).

### Nutrient fluxes

Ambient water  $\text{NO}_x$  and  $\text{NH}_x$  concentrations averaged, respectively, 3 and  $3.5 \mu\text{M}$  in winter and 1 and  $2.5 \mu\text{M}$  in summer. Only effluxes of nutrients were

observed, except for one low density replicate of *Nephtys* sp.  $\text{NH}_x$  effluxes were highest in *Lanice conchilega* treatments (overall average:  $4.17 \pm 0.56 \text{ mmol N-NH}_x \text{ m}^{-2} \text{ d}^{-1}$ ). *L. conchilega* winter effluxes were

very variable and no density pattern was detected. In summer,  $\text{NH}_x$  effluxes tended to increase with this polychaete's densities ( $p = 0.07$ ).  $\text{NH}_x$  effluxes increased significantly with *Abra alba* densities

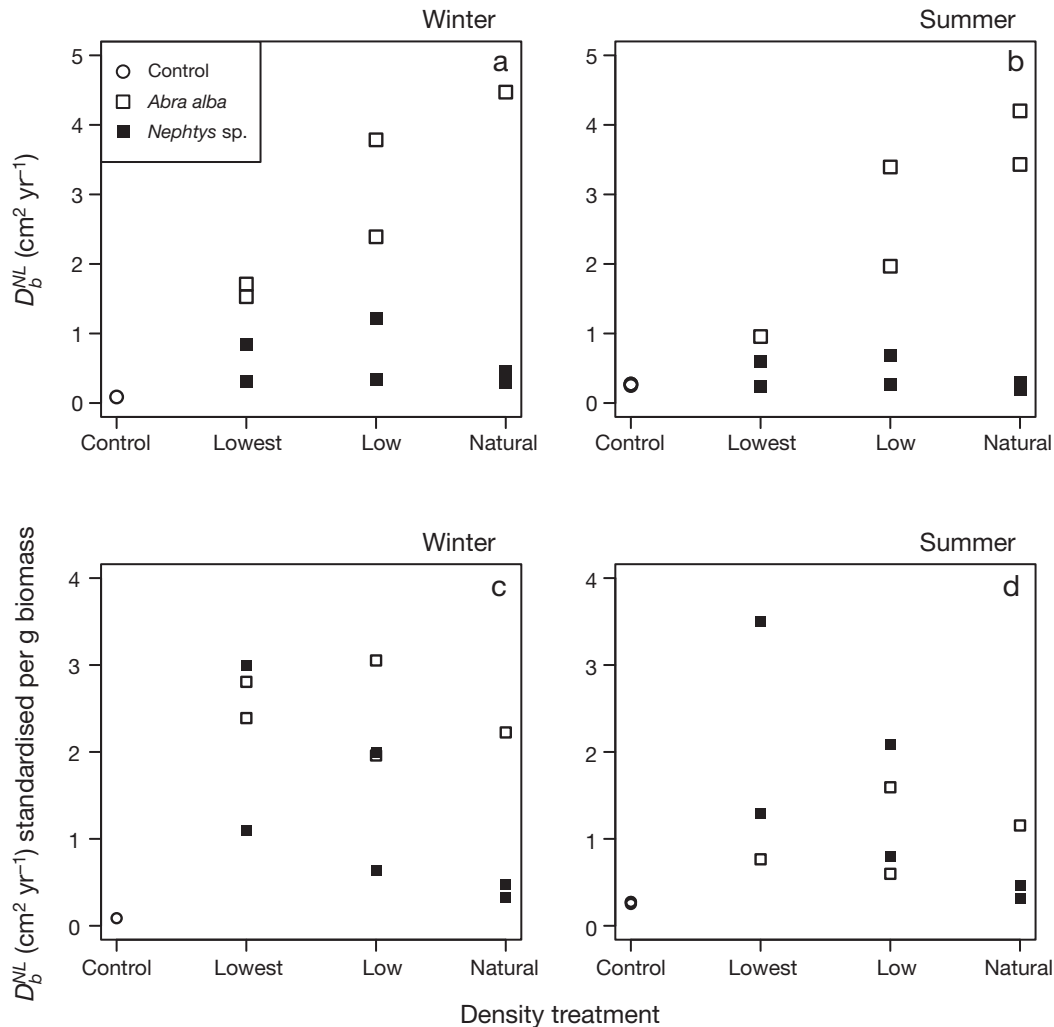


Fig. 2. *Abra alba* and *Nephtys* sp. Bioturbation rate  $D_b^{NL}$  (as  $\text{cm}^2$  of sediment reworked per year) per density level for each species for (a) winter and (b) summer and standardized for biomass (grams AFDW) of added fauna for (c) winter and (d) summer

Table 2. *Abra alba*, *Lanice conchilega* and *Nephtys* sp. Significant simple linear regressions of ecosystem functions on species densities.  $n = 9$ , except for *A. alba* bioturbation ( $n = 5$ ). SCOC: Sediment community oxygen consumption

Species	Time	Ecosystem function	Linear regression analyses			
			a	b	R <sup>2</sup>	p (slope)
<i>A. alba</i>	Winter	Bioturbation	1.27E-3	8.89E-6	0.79	0.028
		$\text{NH}_x$ release	0.19	0.001	0.42	0.030
		SCOC	13.37	0.005	0.74	0.002
		Denitrification	0.08	0.001	0.90	<0.001
<i>L. conchilega</i>	Summer	$\text{NH}_x$ release	2.18	0.002	0.58	0.001
	Winter	SCOC	15.16	0.011	0.50	0.019
	Summer	SCOC	30.27	0.042	0.78	<0.001
	Winter	Denitrification	Quadratic function, see Fig. 5 for formula			
<i>Nephtys</i> sp.	Winter	Denitrification	0.12	0.002	0.78	<0.001

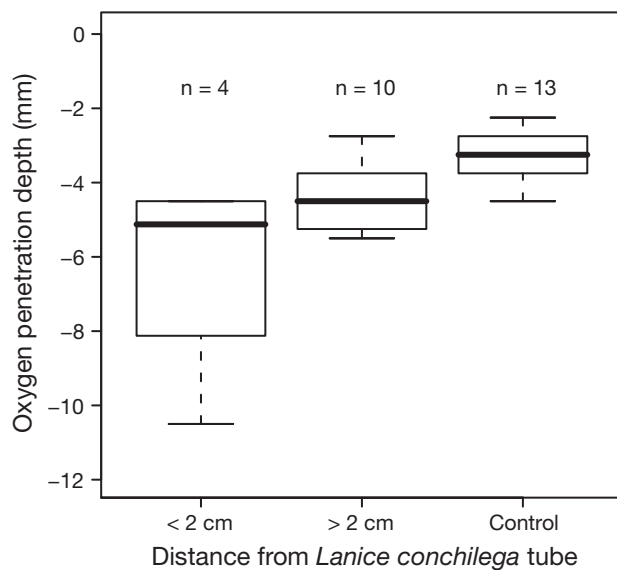


Fig. 3. *Lanice conchilega*. Boxplot indicating the influence of distance from tube on oxygen penetration compared with control: Thick black lines show the median. Box is drawn between the quartiles. Dotted lines extend to the minimum and maximum values

(Table 2) both during winter and summer. There was no linear relationship between  $\text{NO}_x$  effluxes and either densities or species.

### Nitrogen cycling

The mass balance approach yielded good fits to measured oxygen consumption rates and ammonium and nitrate effluxes. Modeled and MIMS based denitrification rates agreed fairly well, except for the *Lanice conchilega* treatments in winter, where measured denitrification was significantly higher than that for modeled denitrification. This difference can be explained either by logistical problems with MIMS measurements or by the lack of steady state conditions. Because of this, we did not test density dependence of denitrification in these *L. conchilega* winter treatments. In winter, measured denitrification increased significantly with densities of *Abra alba* and *Nephtys* sp. (Table 2). In

Table 3. *Abra alba* and *Lanice conchilega*. Significant simple linear regression of sediment community oxygen consumption (SCOC) as a function of species ash free dry weight

Species	Time	Linear regression analyses			
		a	b	R <sup>2</sup>	p (slope)
<i>A. alba</i>	Winter	13.37	0.055	0.74	0.002
<i>L. conchilega</i>	Winter	15.15	0.106	0.51	0.019
	Summer	29.42	0.220	0.83	<0.001

summer, a quadratic function described the pattern of denitrification versus *L. conchilega* densities better than a linear function (Fig. 5), and denitrification was maximal at 'low' density.

Nitrification was calculated from the balance of measured  $\text{O}_2$ ,  $\text{NO}_x$  and  $\text{NH}_x$  effluxes and modeled denitrification. In winter, none of the treatments had higher nitrification rates than control cores (not depicted, but see Fig. 7). In summer, the low and natural *Lanice conchilega* density treatments and lowest and natural *Nephtys* sp. density yielded higher nitrification rates than did controls. *Abra alba* did not exhibit any influence on nitrification rates.

Nitrogen mineralization is the balance of  $\text{NO}_x$ ,  $\text{NH}_x$  and  $\text{N}_2$  effluxes and is proportional to SCOC, apart from denitrification, which contributes a relatively small part to the total mineralization (averaged over all treatments:  $8.9 \pm 1.0\%$ ). SCOC can be attributed to background microbial oxygen consumption, macrofaunal respiration and macrofauna-mediated microbial consumption (Glud 2008, Na et al. 2008). The contribution of the latter can roughly be estimated as the difference between measured SCOC and the sum of background (averaged control) SCOC measurements and estimated macrofaunal respiration derived from biomass data. Positive numbers indicate a macrofauna-enhanced microbial activity (Marinelli & Boudreau 1996, Na et al. 2008).

Species treatments enhanced benthic mineralization differently in winter and summer. In winter, positive numbers were noted in low and natural *Abra alba* and *Lanice conchilega* densities (Fig. 6). In contrast, negative numbers were observed in lowest *A. alba* densities and all *Nephtys* sp. treatments. As these negative values are biologically unlikely, they could indicate estimation errors on faunal respiration. This implies that the positive deviations are too small to conclude that microbial respiration is enhanced convincingly by *A. alba* and *L. conchilega* in winter. In contrast, excess *L. conchilega* respiration in summer was obviously sufficiently large to classify as fauna-mediated microbial respiration. Fauna-mediated microbial respiration increased significantly with *L. conchilega* densities ( $R^2 = 0.77$ ,  $p = 0.01$ ) even up to 100% higher than in the control at one natural density. *A. alba* and *Nephtys* sp. did not seem to mediate microbial respiration in summer.

### DISCUSSION

The use of laboratory controlled microcosms containing defaunated sediment with artificially composed fauna communities is a suitable approach to detect the effects of a single species on biogeochemical cycling. Extrapolating the results of these microcosm experi-



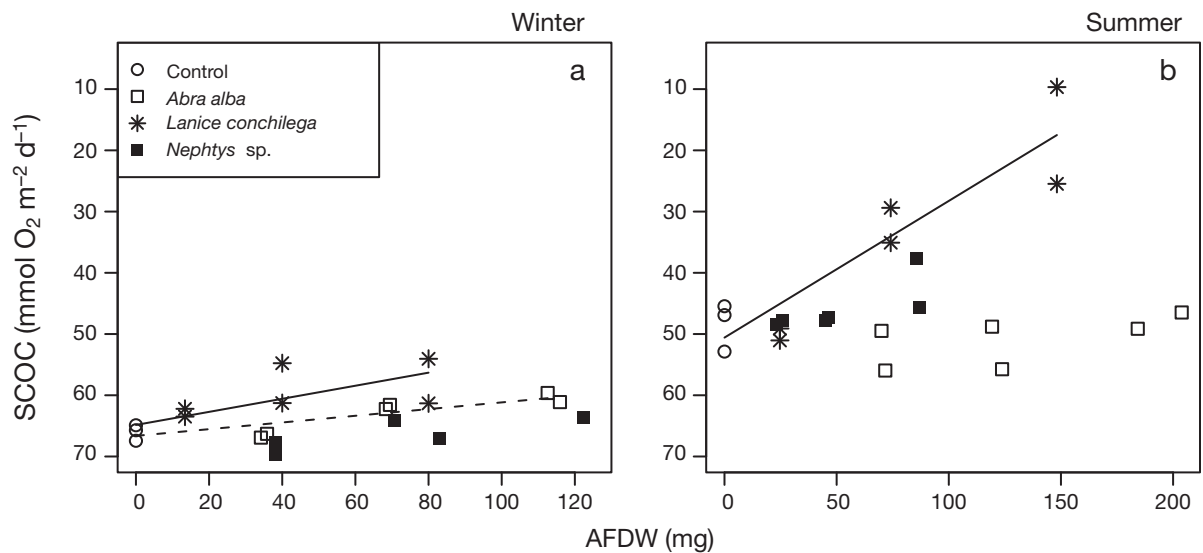


Fig. 4. *Abra alba*, *Lanice conchilega* and *Neptys* sp. Sediment community oxygen consumption (SCOC) as mmol O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> per species as a function of their ash free dry weight (AFDW) for (a) winter (b) summer. Significant regressions of SCOC on *A. alba* densities (solid line) and *L. conchilega* (dashed line) are indicated. Note the x-axis scales are different

ments to broad-scale ecosystem functioning, however, should be done with caution, since the microcosm is a highly simplified and tightly controlled artificial system. Under natural conditions, the role of each of the isolated species in this study will rather be a function of the complex interactions of competition for space and food (Ieno et al. 2006), macrofauna patchiness (Barry & Dayton 1991, McIntosh 1991), sediment dynamics and water flow (Biles et al. 2003, Tengberg et al. 2003 and references therein).

Nevertheless, microcosm experiments provide a mechanistic understanding of ecological processes, thereby supplying mathematical models with the necessary biological information for parameter selection and estimation and influencing the development of theories that are of fundamental importance to address global ecological issues (Benton et al. 2007). Therefore, they are a natural step between mathematical modeling and necessary empirical tests in large-scale marine systems (Oksanen 2001).

Benthic activity in the North Sea is influenced by the seasonal input of organic matter (Boon & Duineveld 1998), but because benthic chl *a* concentrations in our experiments were similar in summer and winter, the fresh, highly labile organic matter was probably already mineralized (van Oevelen et al. 2009). Nevertheless, higher sediment–water exchange fluxes were observed in the microcosms in summer, in large part due to macrofaunal stimulation. The higher macrobenthic activity in summer can thus be explained by the higher temperature and better condition of the animals, i.e. they were better fed at the time of field sampling.

#### MECHANISTIC EXPLANATION OF THE RELATIONSHIP BETWEEN FUNCTIONAL TRAITS AND ECOSYSTEM FUNCTIONING

Benthic mineralization makes nutrients available to the pelagic algae (Soetaert et al. 2001). Deposited particulate OM is first mineralized to carbon dioxide and ammonium (Fig. 7). In the presence of oxygen, ammonium can subsequently be oxidized to nitrite and nitrate, which serve as substrates for denitrification under anoxic conditions. The nitrogen gas resulting from

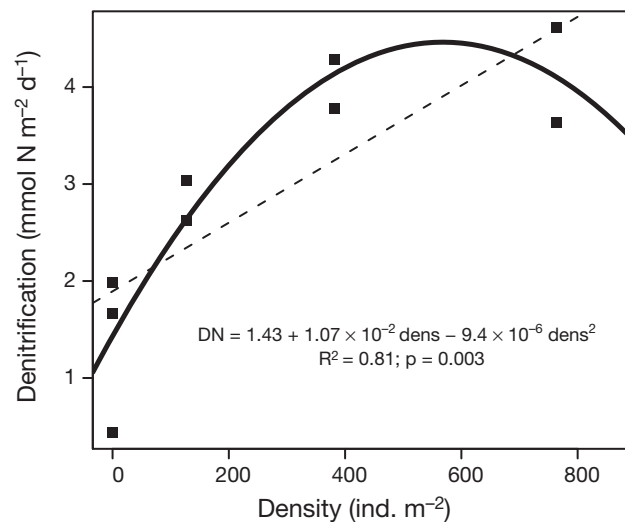


Fig. 5. *Lanice conchilega*. Denitrification as a function of densities in summer. Lack of fit test indicated a better fit by quadratic rather than linear function

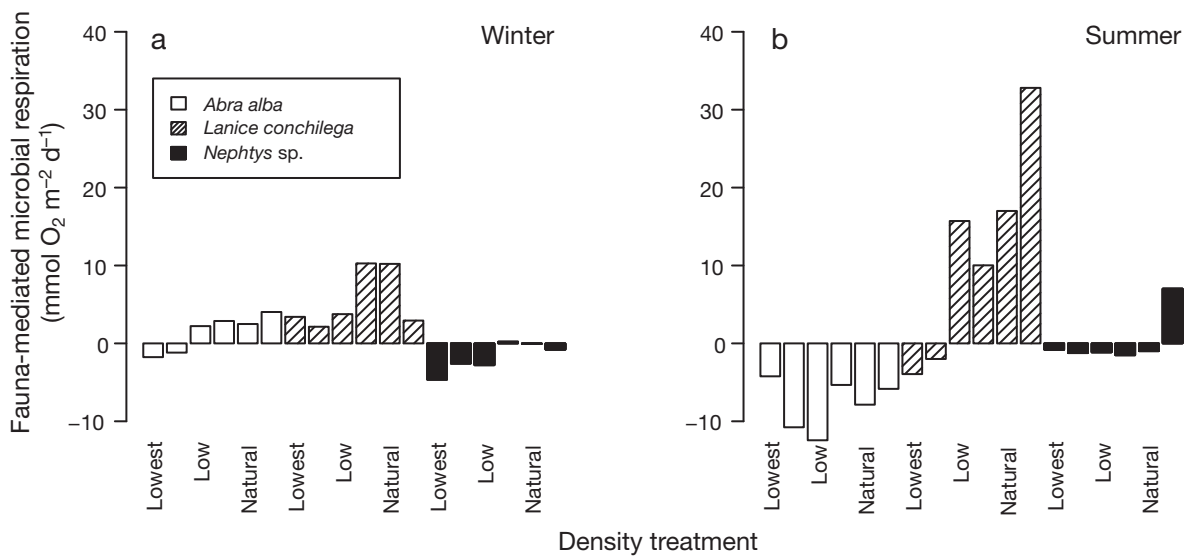


Fig. 6. *Abra alba*, *Lanice conchilega* and *Nephtys* sp. Fauna-mediated microbial respiration (for (a) winter and (b) summer) for increasing densities of all species in  $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . Values are calculated as measured sediment community oxygen consumption (SCOC) minus predicted SCOC

this process is not available to most micro-organisms and eventually escapes to the air. This transfer of fixed nitrogen to nitrogen gas is important in coastal systems, because it counteracts N eutrophication.

In the absence of fauna (control cores), SCOC was 2.0 times higher in summer compared with winter (Fig. 7a), which can be primarily attributed to a temperature effect ( $Q_{10} = 2.4$ ), since sediment chl *a* concentrations were similar. The higher mineralisation in the summer experiment (reflected in SCOC) resulted in more ammonium production, higher  $\text{NH}_x$  effluxes and higher nitrification rates. The resulting nitrate was partly lost to the water column as the nutrient concentrations in the overlying water were low. Nevertheless, sufficient nitrate was available in the sediment to fuel denitrification.

Bio-irrigation and bioturbation influenced these processes and the stimulation by the 3 studied species was different, which can be explained by their behaviour. *Abra alba* resides in the upper 3 to 4 cm of the sediment (Degraer et al. 2006), extending its siphons to the sediment surface. The oxygenated water transported through the siphons is not in direct contact with the surrounding sediment, but the siphonal channels of *Abra* spp. are probably important in bringing oxygen to deeper strata (Wikander 1980). Although the sediment was thoroughly reworked in both experiments and the gray color indicated reoxidation of reduced metabolites, the oxygenated layer was not extended compared with the control (Fig. 7b). This is reflected in the observed fluxes: oxygen consumption at natural *A. alba* density was comparable with controls in both

winter and summer experiments, while ammonium effluxes were enhanced by a factor 3.3 in summer, at the expense of nitrification and denitrification.

Similarly, Davis & Miller (1979) expected an enhanced oxygenation through burrow walls of *Nephtys* sp. The sediment surrounding burrow linings was indeed gray, but overall oxygen penetration depth in *Nephtys* sp. cores did not differ from the control depth. SCOC was 2.0 times higher in summer compared with winter, while ammonium effluxes were 3.2 times higher (Fig. 7d). Given that only 20% of the ammonium efflux can be attributed to animal excretion (Christensen et al. 2000), the greater ammonium effluxes in the cores must have been supported by enhanced ammonium regeneration, at the same time fuelling nitrification and indirectly denitrification. Our observations of *Nephtys* sp. barely bioturbating and enhancing sediment–water fluxes deviate somewhat from expectations based on *Nephtys* life history in natural conditions. *Nephtys* sp. is an active predator (e.g. Fauchald & Jumars 1979), able to cope with high environmental stress (Arndt & Schiedek 1997). Food supply greatly increases bioturbation and ventilation by the polychaete *Nereis diversicolor* (Kristensen 1989, Nogaró et al. 2008). When no food is found, *N. diversicolor* returns to its burrow and resumes ventilation activity. As no extra food was added to the experimental cores, we suggest that the defaunated sediment deprived these predators of suitable prey and made them adopt an energy saving behaviour, especially in winter.

In contrast to *Abra alba* and *Nephtys* sp., *Lanice conchilega* generated the highest fluxes, in winter and

summer (Fig. 7c). This might be explained by the fact that *A. alba* and *Nephtys* sp. do not actively irrigate their burrows/feeding pits; hence, their influence on measured fluxes is limited. Similarly, Mermillod-Blondin et al. (2004) and Michaud et al. (2005, 2006) observed fairly small effects of natural densities of the bivalves *Cerastoderma edule* and *Macoma balthica*, also biodiffusers, on sediment–water fluxes.

Oxygen penetration depth in *Lanice conchilega* microcosms was larger than those for the other species and control treatments. Varying penetration depths were observed, depending on the distance to the polychaete tube. Even though *L. conchilega* feeds via suspension–deposit feeding and respires using gills at its anterior end, the species ventilates its tube for 1.5 min every 4 min, transporting about  $8 \mu\text{mol O}_2 \text{ ind.}^{-1} \text{ d}^{-1}$  into the sediment (Forster & Graf 1995), leading to an oxic layer surrounding the tubes and with oxygen concentration reaching 20% of air saturation up to the 55 mm depth. This larger oxic volume might stimulate microbial mineralization and generation of ammonium. Combined with the strong pumping behaviour of this

species, this clarifies the very high ammonium effluxes in treatments of *L. conchilega*. According to the mass balance estimates, these ammonium effluxes were too large to sustain nitrification and denitrification as high as observed in the control microcosms in winter. Moreover, modelled denitrification rates were far below rates measured by MIMS. Although MIMS-based denitrification has its limitations (see 'Materials and methods'), we believe that the discrepancy can be attributed to a lack of steady state conditions and thus the applicability of the mass balance approach adopted. Introducing bio-irrigators to a defaunated sediment column will delay reaching a steady state situation (Banta et al. 1999), which is typically characterised by very high ammonium effluxes due to the high amount of labile OM that becomes available (Na et al. 2008). However, ammonium effluxes and oxygen influxes were better balanced in the summer experiment. Intermittent ventilation patterns may promote variable  $\text{O}_2$  conditions in and around the tubes (Kristensen 1988) with repercussions on other fluxes. The ammonium that accumulated in and around the *L. conchilega* tubes was presumably

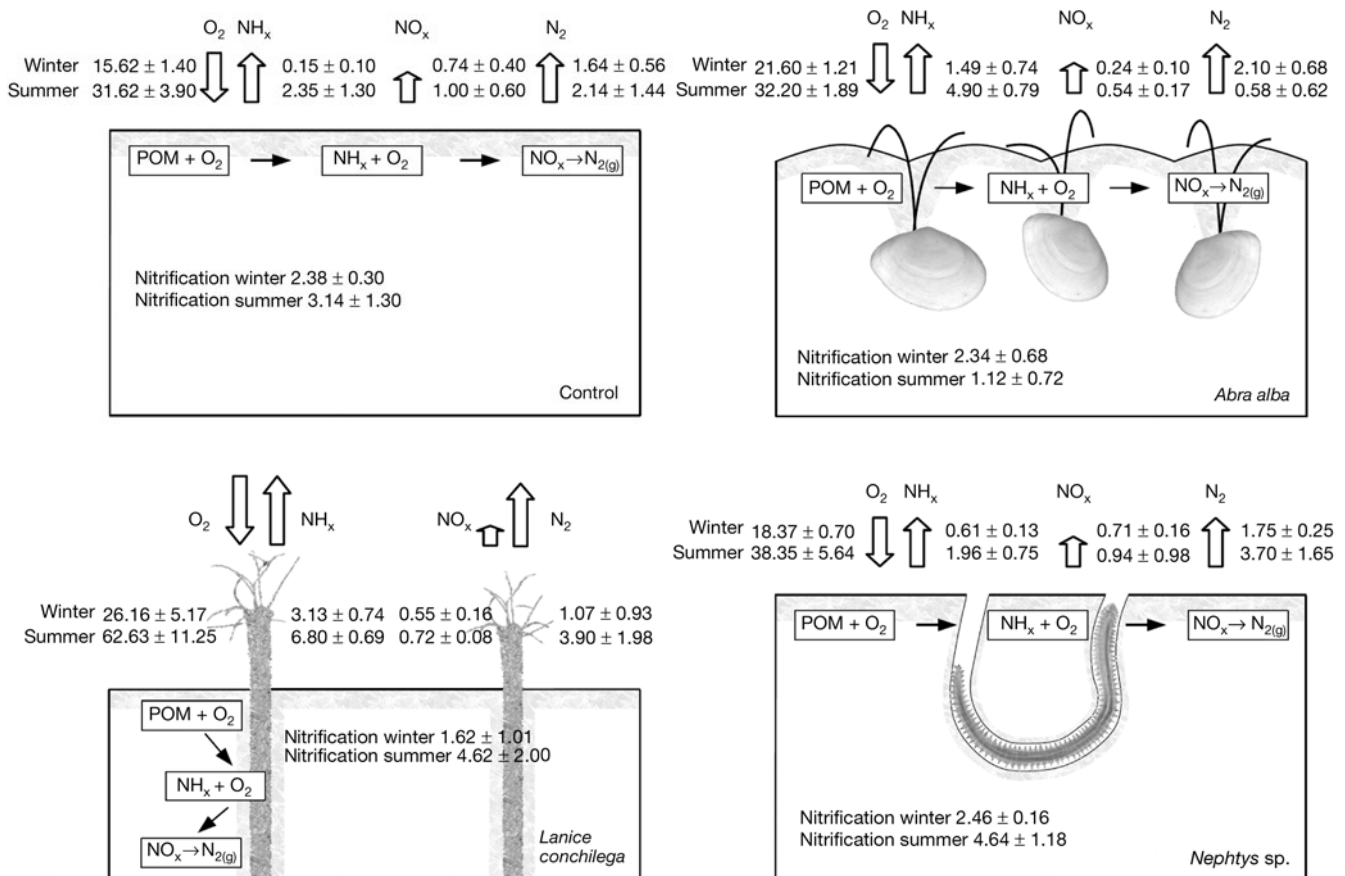


Fig. 7. *Abra alba*, *Lanice conchilega* and *Nephtys* sp. Schemes of mass balanced processes at natural density for each species treatment.  $\text{O}_2$  fluxes are indicated in  $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ .  $\text{NH}_x^-$ ,  $\text{NO}_x$  fluxes, nitrification and  $\text{N}_2$  fluxes are given in  $\text{mmol N m}^{-2} \text{ d}^{-1}$  (multiply by 0.8 to express in  $\text{mmol C}$  or  $\text{O}_2 \text{ m}^{-2} \text{ d}^{-1}$ )

nitrified to nitrate during a resting period of the polychaete until oxygen was depleted, and nitrate denitrified to  $N_2$ . In the next irrigation period, this  $N_2$  gas and remaining ammonium were flushed out and fresh oxygenated water drawn into the sediment. In cores with a natural density of *L. conchilega*, benthic mineralization in summer was doubled compared with the control. This is of the same order of what has been found by Kristensen & Blackburn (1987) for *Nereis virens* and by Banta et al. (1999) for *Arenicola marina* and confirms that bio-irrigation influences mineralization and N cycling more drastically than bioturbation (Mermillod-Blondin et al. 2004).

### Importance of density

For many ecosystem processes there were no significant density effects, but there were some notable exceptions. In these cases decreases in densities may have implications for ecosystem functioning.

Bioturbation rate and ammonium effluxes increased in both experiments with density of *Abra alba*. The intense bioturbation and thus downward transport of OM is important for the food such as bacteria and meiobenthos (Levin et al. 1997, Witte et al. 2003) and microbial decomposition and mineralization processes that consume oxygen and release  $NH_x$  (Herman et al. 1999, Thrush et al. 2006). The standardized  $D_b^{NL}$  of *A. alba* was higher in winter than in summer. Considering the lower biomasses in winter, this can indicate a higher bioturbation activity of the invertebrates in winter. Another possibility is that *A. alba* body mass does not significantly affect the individual's bioturbation activity, which mainly results from siphon movements.

*Nephtys* sp. populations did not rework the sediment actively, but when standardised to biomass, the bioturbation rate dropped with increasing densities. This has also been observed for *Nereis diversicolor* (Duport et al. 2006) and has been linked to territorial behaviour (Miron et al. 1992).

Oxygen penetration tended to increase with *Lanice conchilega* densities. We did not manipulate densities up to 5000 ind.  $m^{-2}$  as found in reefs (Rabaut et al. 2009) because of experimental restrictions. Nevertheless, at a certain tube density the oxygenated zones will overlap; thus, even small reefs of *L. conchilega* can enhance oxygen penetration on a relatively large scale. Interestingly, denitrification in *L. conchilega* treatments reached a maximum at 'low' density. Aller (1988) showed that denitrification potential initially increases with density and then drops at a certain tube density. This is explained by over-oxygenation causing large reduction of sediment areas for coupled nitrification/denitrification. However, this should be further

explored with higher *L. conchilega* densities, as it may show a different pattern at reef dimensions.

Our approach involved natural and lower densities rather than artificially high densities, although use of the latter would facilitate observation of the effect. We believe that comparing the effects between natural populations of species has the advantage of estimating their relative importance in the field. As such, we can conclude that the natural density of *Abra alba* in the cores contributed more to sediment reworking than the natural *Nephtys* sp. density, which is related to a higher density and biomass. Finally, though at a lower natural density and biomass, adding *Lanice conchilega* to microcosms caused a more pronounced influence on fluxes than did *A. alba*.

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