CHAPTER 3

Contrasting macrobenthic activities differentially affect nematode density and diversity in a shallow subtidal marine sediment

ABSTRACT

By bioturbating and bio-irrigating the sea floor, macrobenthic organisms transport organic matter and oxygen from the surface to deeper layers, hereby extending the habitat suitable for smaller infauna. Next to these engineering activities, competition, disturbance and predation may also affect the spatial distribution of these smaller organisms. In a controlled laboratory experiment, we studied the effect of 3 functionally different macrobenthic species on the vertical distribution of nematodes. *Abra alba,* a suspension–deposit feeding bivalve reworking the sediment randomly, *Lanice conchilega,* a suspension-deposit feeding, tube-irrigating polychaete and *Nephtys hombergii,* a burrowing predatory polychaete were added in single–species treatments to sediment from a coastal subtidal station in the Belgian part of the North Sea, sieved (1 mm) to remove macrofauna. After 14 d, the control treatment without macrobenthos was found to be detrimental to nematode density and diversity, which points to the importance of macrobenthic engineering to sustain the smaller components of the food web. Nematode densities were highest at the sediment surface in all treatments, but subsurface density peaks were observed in *A. alba*[2] (to 4 cm depth) and *L. conchilega*[3] (to 7 cm depth) microcosms. In the *A. alba* treatment, the dominant non-selective deposit

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[2] 1.8 to 4.8x higher nematode densities in these layers of the *Abra alba* microcosms compared to the nematode densities in the same depth layers of the control microcosms

[3] 3.4 to 5.4x higher nematode densities in these layers of the *Lanice conchilega* microcosms compared to the nematode densities in the same layers of the control microcosms

feeders and the epistrate feeders shifted downwards probably to avoid disturbance and exploitative competition by the bivalve siphons at the surface, while they might have benefited from the faecal pellets deposited in the subsurface. In the *L. conchilega* treatment, several dominant species were redistributed over depth layers, indicating polychaete-mediated habitat extension from surface into depth. Nematode communities seemed hardly affected by the presence of *N. hombergii*. These results reveal that functionally contrasting macrobenthic engineering effects shape nematode communities in different ways which may maintain the role of nematodes in ecosystem functioning. The present study therefore highlights the need for conservation of macrobenthic functional diversity.

Key words: Bioturbation · Macrobenthos · Ecosystem engineering · Nematodes · Vertical distribution · Diversity · Density

INTRODUCTION

One of the major goals of ecology is to understand the distribution of species, populations and communities. Disentangling the drivers of these distributions is challenging since a wide variety of biotic and abiotic factors are recognised as being important in shaping biological communities. Gaining insight into these complex mechanisms has now become more pressing than ever in the light of changing habitats and increasing rates of species extinctions. A key pathway by which one organism can affect others is by physically changing its abiotic environment, so called ecosystem engineering (Jones *et al.* 1994). For example, macrobenthic organisms that inhabit marine soft sediments alter the distribution of high quality organic matter (Graf 1989; Levin *et al.* 1997), oxygen and toxic metabolites in the sediment (Kristensen and Kostka 2005) through bioturbation and bio-irrigation activities (Aller 1988; Meysman *et al.* 2006a) which, in turn, may influence the distribution of organisms that are too small to "engineer" their own habitat, *e.g.* nematodes (Reise 1985) or bacteria (Mermillod-Blondin *et al.* 2004). The ecosystem engineering concept however, is controversial in that all organisms are affected by and change their abiotic environment to some extent (Reichman and Seabloom 2002). Consequently, the utility of the concept requires explicit

effects of the ecosystem engineer on other organisms that differ from direct biotic interactions (Hastings *et al.* 2007).

Nematode vertical distribution in marine soft sediments is tightly coupled to (1) the prevailing geochemical properties, such as hydrogen sulfide and oxygen concentrations (*e.g.* Platt 1977; Hendelberg and Jensen 1993; Wetzel *et al.* 1995), and (2) food availability in the sediment (Franco *et al.* 2008a). These factors can be altered by bio-irrigation and bioturbation, not only by direct transport of solids and solutes, but also by stimulating biogeochemical processes along the burrow walls (Mermillod-Blondin *et al.* 2004). The sediments surrounding burrows likely support microbial communities that differ from those in the surficial sediments (Kristensen and Kostka 2005) and may therefore diversify the menu for nematodes. Further, macrobenthic faecal casts give rise to high bacterial activity due to a greater concentration of fine particles, rich in organic carbon and nitrogen, than in the surrounding sediment (Reise 1985). In addition, macrobenthos may affect nematode communities positively, by the construction of physical structures (*e.g.* polychaete tubes) that provide shelter from predation (Zühlke *et al.* 1998), or negatively, by depleting their food resources (Ólafsson *et al.* 1993), and by direct physical disturbance due to regular bioturbation (Austen and Widdicombe 1998; Schratzberger and Warwick 1999).

Studies investigating the link between macro– and meiofauna (*e.g.* nematodes) are numerous (reviewed by Ólafsson 2003; Dashfield *et al.* 2008; Bouchet *et al.* 2009) but evidence for the biogeochemical engineering effects of macrofauna on nematode distribution remains surprisingly scarce: the engineering mechanisms involved (*i.e.* altered environmental variables) are not always studied in detail (Ólafsson *et al.* 1993; Austen *et al.* 1998; Austen and Widdicombe 1998; Austen and Thrush 2001) and therefore clear proof of interactions between macrobenthos, environmental variables and meiofauna are often lacking. Ólafsson (2003) pointed out that enhancing the resolution in data collection, *e.g.* by taking the vertical distribution of the sediment into account and working at low(er) taxonomic levels, is crucial in determining macrofauna effects on nematodes. Moreover, the examination of whether macrofaunal species with a different functionality (in terms of bioturbation) differently affect nematode communities may help to identify the engineering mechanisms involved. Macrobenthic functional traits in terms of bioturbation are manifold and comprise (1) biodiffusers that rework the sediment at random, (2) gallery-diffusers that excavate and actively irrigate

burrows, (3) upward-conveyors that remove sediment at depth in the substratum and expel it at the sediment–water interface, (4) downward-conveyors that cause "active" transport of sediment through their gut from the sediment–water interface to their egestion depth and (5) regenerators that dig out sediment at depth and move it to the surface, where it is washed away and replaced by sediment from the surface (Gérino *et al.* 2003; Michaud *et al.* 2006). These differing traits have all been shown to make the biogeochemical sediment environment more heterogeneous (Mermillod-Blondin *et al.* 2004; Michaud *et al.* 2006; Braeckman *et al.* 2010) and thus potentially affect nematode vertical distribution.

The present work explores for the first time the nematode community response to functionally contrasting macrobenthic activities in shallow subtidal sediments, both on a detailed taxonomic level and with respect to their vertical distribution. We introduced 3 functionally different macrobenthic species in single-species treatments into microcosms containing (subtidal) sediment with the same natural nematode communities but devoid of the natural macrobenthic population. The three species are dominant representatives of the *Abra alba–Kurtiella bidentata* community in the Belgian part of the North Sea (Van Hoey *et al.* 2004). Both the bivalve *Abra alba* and the polychaete *Nephtys hombergii* rework the sediment at random (*i.e.* they are biodiffusers), though the first species is a suspension-deposit feeder while the latter is a predator. The tube-irrigating, suspension–deposit feeding polychaete *Lanice conchilega* is sedentary and has limited impact on sediment turn-over once the tubes are established. Its piston–pumping (Forster and Graf 1995) stimulates microbial activity and enhances benthic mineralisation, whereas the biodiffusers *A. alba* and *N. hombergii* do not irrigate their burrows or feeding pits, resulting in a limited stimulation of benthic mineralisation rates (Braeckman *et al.* 2010).

We tested whether the presence of macrobenthos affects the vertical profile of environmental variables and nematodes, and whether different functional groups of macrobenthos have a contrasting effect on the vertical profile of environmental variables and nematodes.

MATERIAL AND METHODS

Study site and sampling

The experimental set-up required sediment containing the natural vertical distribution of nematodes excluding abundant macrofauna. Therefore, sediment from a sandy coastal station off the coast of Oostende (51°19.27'N, 2°52.09'E, 18 m depth, 9 km offshore) was collected with a Reineck boxcorer from the *RV* Zeeleeuw. Sediment median grain size at the study site is 215 ± 0.22 μm with a mud fraction (<63 μm) of 5.8 ± 0.2%. The macrobenthic community present belongs to the *Abra alba-Kurtiella bidentata* community (Van Hoey *et al.* 2004) . The sediment was sliced into 0-1 cm, 1-3 cm, 3-5 cm, 5-8 cm, 8-end cm sections, wet sieved to remove macrofauna (> 1 mm) and brought to a temperature controlled laboratory (18 °C) 7 days before the start of the experiment (Day -7). The naturally oxygenated 0-1 cm section was aerated overnight. During this sampling campaign, 3 cores of 3.6 cm internal diameter [i.d.], obtained from replicate deployments of the Reineck boxcorer, were sliced in 1 cm sections to serve as a field control [FC]. Macrobenthos inhabiting these FC slices was retained on a 1 mm mesh. On Day -6, the sediment column (8 cm) was reconstructed by stacking subsequent sediment horizons in cylindrical microcosms of 10 cm i.d. The microcosms were left to acclimatise in the dark for 4 d at 18°C, covered with 20 cm of natural seawater of salinity 32.

Lanice conchilega was collected in the intertidal area by means of metal frames (Rabaut 2009) and subsequently introduced into the microcosms within its tube as described in Ziegelmeier (1969) at a natural density of 637 ind.m^{-2} (corresponds to 5 animals introduced into each microcosm). To check the fitness of the animals, the fringed tube end was cut after introduction to the sediment. The next day, all animals had rebuilt a new crown of fringes. *Abra alba* and *Nephtys hombergii* were sampled from the subtidal with the *RV* Zeeleeuw and introduced at a density of 764 ind.m^{-2} (7 animals introduced) and 382 ind.m^{-2} (3 animals introduced) respectively which is within the ranges of their natural density (Degraer *et al.* 2006). *N. hombergii* and *A. alba* specimens that did not burrow within 30 min. were replaced by more active individuals.

Experimental set-up and slicing

Twelve microcosms were attributed to 4 experimental treatments in triplicates: 3 experimental control [C] microcosms where reconstructed sediment was incubated

without macrofauna; three microcosms each with *Abra alba*, *Lanice conchilega* and *Nephtys hombergii.* Three additional reconstruction controls [RC] served as a procedural control for possible disturbance during and after microcosm reconstruction (homogenisation and compaction of the sediment). Microcosms were incubated at constant room temperature of 18°C for 14 d. The overlying water in the microcosms was continuously aerated and replaced every 2 d to avoid accumulation of NH_x. The three RC were sliced on Day 1 in 1 cm sections. On Day 14, all experimental microcosms were sliced in defined vertical sections as described above ('Study site and sampling'), but with higher resolution in the first 2 cm for closer inspection of nematode species response (0-0.5, 0.5-1, 1-1.5 and 1.5-2 cm). The sediment from each slice was homogenised and subsamples were taken for nematode density and community analysis (5 ml in 0.5 cm slices and 10 ml in 1 cm slices) and stored in a buffered 4% formalin solution. Meiofauna was extracted by centrifugation with Ludox (Heip *et al.* 1985). All nematodes were hand-picked, mounted onto slides and identified to genus or species level according to the pictorial key of Warwick *et al.* (1998). Sediment subsamples (10 ml) were dried at 60°C and median grain size and silt content was determined with a Malvern Mastersizer using laser diffraction. Sediment subsamples for pigment analyses (10 ml) were stored at -80°C until analysis and sediment water content was calculated from the difference in weight between frozen and freeze-dried sediment. The freeze-dried sediment was extracted with 10 ml 90% acetone and pigment (chl-*a* and phaeophytin-*a* and phaeophytin-*a*-like) concentrations in the supernatant were determined using HPLC (Gilson) analysis (Wright and Jeffrey 1997).

Data analysis

A fully crossed 3-factor design was performed in PERMANOVA with random factor replicate [Rep] nested in the fixed factor treatment [TR], next to the fixed factor slice [Sl]. The interaction term TRxSl informs us about the difference in depth profiles of nematode uni– or multivariate measures or environmental variables among treatments. Since a PERMANOVA test can show significant differences between groups, but does not distinguish between a difference due to location (factor effects) or dispersion (variance), homogeneity of multivariate dispersion was tested with PERMDISP, using distances among centroids calculated both within Rep(TR) groups (averaged depths) and in TRxSl

(averaged replicates) groups. The PERMDISP-test was never significant, indicating equally dispersed distances to centroids, hence a difference due to location (TRxSl interaction, hence "profile"). In case of significant TRxSl interactions, pairwise tests of TR within TRxSl were performed to investigate in which slice the treatments differed or vice versa. With only significant single factor effects, pairwise tests within each factor were carried out. Because of the restricted number of possible permutations in pairwise tests, p-values were obtained from Monte Carlo samplings (Anderson and Robinson 2003). The 3-factor design was used for both multivariate and univariate analyses since PERMANOVA allows us to perform univariate ANOVAs with p-values obtained by permutation, thus avoiding the assumption of normality. A Euclidean distance and Bray-Curtis based resemblance matrix was used for univariate and multivariate measures. The TR effect on the vertical structure of the nematode community was tested on (1) standardised (to account for differences in total nematode abundances among treatments) and square root-transformed data (to reduce the importance of dominant species) and on (2) raw data to take differences in species/genus abundance into account.

First, the difference between FC, RC and C profiles in terms of nematode density and community composition was tested. Further analyses considered experimental treatments only. The effect of macrobenthos on the totals and depth profiles of densities, diversity indices (species richness (S); evenness (J'), Hill's indices N_1 and N_∞) and depth profiles of environmental variables was analysed. Sample sizes varied between levels of depth (5 ml for for 0.5 cm slices, 10 ml for 1.0 cm slices). As samples vary proportionally, a non-linear relationship between sample volume and number of species cannot be excluded. Thus for all statistical analyses on nematode community structure and diversity indices that include depth horizons through the whole core (0 to 8 cm), 0.5 cm-slice samples were combined into 1.0 cm-slices to give comparable sample sizes, and separate analyses were run on the upper 2 cm samples at 0.5 cm discrimination.

Within the multivariate analyses, a SIMPER analysis was selectively carried out on the slices that differed significantly among treatments in nematode community composition. A second-stage MDS plot with additional ANOSIM visualises the correlation between the vertical profiles of the nematode community composition. DISTLM (DISTance Based Linear Models) is a routine for analysing and modeling the relationship between a multivariate data cloud and one or more predictor variables. This analysis was carried out to identify the environmental parameter (1 cm – scale) with the greatest influence on the

variance distribution of the nematode community composition. After elimination of highly correlated (*i.e.* |r| > 0.8) variables (*i.e.* % silt with median grain size), this analysis was performed on the normalised variables chl-*a*, phaeophytin-*a*, phaeophytin-*a*-like pigment, %silt, and water content using a global BEST selection procedure with Bayesian Information Correction (BIC).. A generalised linear model (GLM) calculated the amount of variation in the nematode univariate measures (Poisson distribution was assumed) explained by combinations of environmental variables. If the residual variance exceeded the degrees of freedom, the data were "overdispersed", and one of the assumptions of GLMs was violated. In this case, a compensation of the significance values was included in the models (Breslow 1984). The GLM accompanying adjusted D^2 is reported, which is a measure - equivalent to adjusted R^2 in Least Squares models - that resembles the fit of the model and increases with an increasing number of observations (n) or a decreasing number of parameters in the model (Guisan and Zimmermann 2000).

All analyses were performed within PRIMER v6 with PERMANOVA add-on software (Clarke and Gorley 2006; Anderson *et al.* 2008). Exceptions were the one-way ANOVAs on the difference in total nematode densities and diversity indices among treatments with additional Tukey HSD-post hoc tests and the GLM carried out in R 2.6.0 software (www.r-project.org). Results are expressed as mean values ± SE of triplicates.

RESULTS

Procedural controls

Macrobenthos resident in the FC was very scarce, consisting of 1.3 ± 0.4 *Nephtys* sp. juveniles 10 cm² and 0.3 ± 0.4 *Aonides* sp. juveniles 10 cm² concentrated in the upper 2 cm.

The total nematode density in the FC, RC and C did not differ significantly (1-way ANOVA: $F_{2,6}$ = 2.71, p = 0.15). The vertical profiles of the nematode densities however, were distinct (Figure 1, Appendix Table 1). Nematode densities in the FC increased gradually to a depth of 3 cm below which they decreased again with depth, while in both C and RC this gradual pattern was replaced by a sharp decline in nematode densities below 1 cm. Significant differences between C and RC were observed in the deepest sediment layers: the RC had a significantly higher nematode density at depth than the FC and C. Also in terms of the nematode community structure, in the FC the vertical structure was distinct

from the C, but mainly in terms of abundance of dominant species (as detected by the higher pseudo-F value in the PERMANOVA-analysis on raw data *vs.* standardised and square root-transformed data) (Appendix Table 2). The differences between FC and RC must be linked to manipulations during reconstruction, *i.e.* sieving and homogenising, and sediment compaction due to removal of particles and fauna > 1 mm. In contrast, apart from a lower nematode density in the deepest layer in C probably due to anoxia-related mortality, nematode communities in C did not differ from those in RC. Thus, maintaining the microcosms in experimental conditions did not introduce large differences into the systems and the patterns observed among experimental treatments can be linked to the effect of macrobenthic presence.
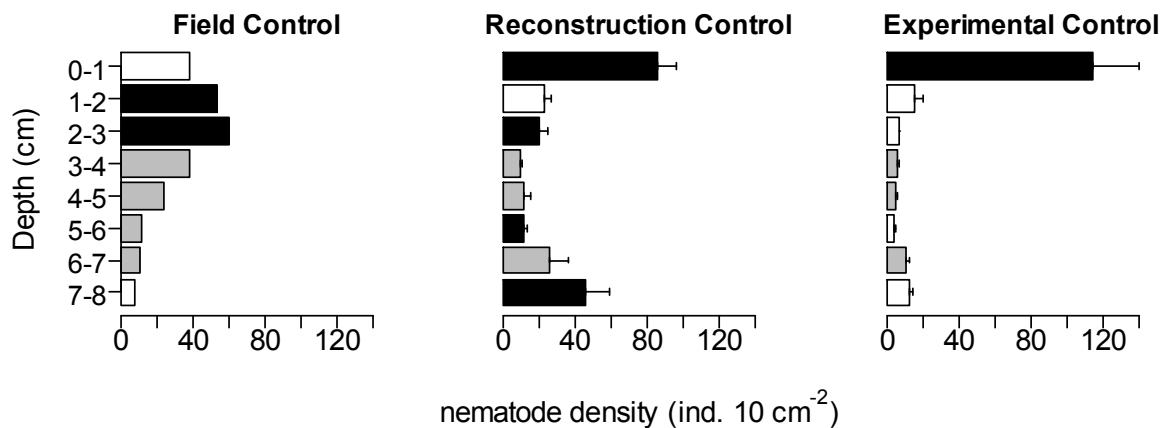

Figure 1. Nematode density profiles in Field, Reconstruction and Experimental Controls (from left to right). The higher (black bar) and lower (white bar) nematode density of eachpair according to pairwise tests; and slices not detected as significantly different from other slices(grey bar) are indicated. Error bars indicate SE

Experimental treatments

Survival of added macrobenthos

All added macrobenthos except 1 *Abra alba* individual were recovered live on slicing. *A. alba* individuals were found between 1.5 and 7 cm depth and mainly (77%) between 2 and 4 cm depth. *Nephtys hombergii* resided between 1 and 6 cm depth, with 76% in the 2 to 5 cm layer. *Lanice conchilega* tubes extended to the very bottom of the microcosms (8 cm).

Environmental variables

The macrobenthic functional groups did not affect the depth profiles of sediment median grain size and silt content (*i.e.* no interaction effect TRxSl) and there was only a significant 'Slice' effect (Table 1): sediment median grain size was smaller and silt content higher in the top centimetre in all experimental treatments. Water content of the sediment was significantly altered by the macrobenthic treatments (Table 1, Figure 2) and was significantly enhanced in the *Lanice conchilega* treatment in the upper 0.5 cm (compared to the *Abra alba* treatment) and in the 2-3 cm layer (compared to control).

The water content in the *A. alba* treatment was higher in the 2-3 cm and 6-7 cm layers (compared to control) and 4-6 cm layer (compared to *L. conchilega*) (Appendix Table 3). Chl-*a* profiles were also significantly structured by the macrobenthic treatments (Table 1, Figure 2): the chl-*a* content of the *A. alba* treatments was depleted in the upper 1.5 cm and also the 4-5 cm layer of this treatment had a lower chl-*a* content than the same slice in the *L. conchilega* treatment (Appendix Table 3). The profile of the chl-*a* degradation products differed among treatments for phaeophytin-*a*, but not for phaeophytin-*a*-like pigment (Table 1). Phaeophytin-*a* content followed a similar pattern to chl-*a*, although less pronounced, being significantly lower in the surface layer of the *A. alba* treatment.

Nematode density and diversity

The average total number of nematodes per treatment (over entire microcosm of 8 cm depth) was 233 ± 18 ind. 10 cm^{-2}. Nematode densities differed among treatments (Appendix Table 4) and were significantly higher in *Lanice conchilega* microcosms than in control microcosms (Tukey HSD post-hoc: $p = 0.033$). As the proportion of juveniles was equal in all treatments, this difference should be attributed to mortality in the controls. The macrobenthic species belonging to 3 different functional groups had contrasting structuring effects on the vertical distribution of the nematodes (Table 1, Figure 3). Whereas nematode densities declined rapidly below 0.5 cm in the control treatment, significant subsurface peaks were observed (Figure 3, Appendix Table 5) in *Abra alba* (0.5 to 1.5 cm; 2 to 4 cm) and *Lanice conchilega* (1 to 1.5 cm, 4 to 5 cm, 6 to 7 cm) treatments. In the *Nephtys hombergii* microcosms, nematodes were likewise found somewhat deeper

than in the control microcosms (2 to 3 cm, 3 to 4 cm, 6 to 7 cm). The nematode abundance in the upper 0.5 cm in the *A. alba* treatments was significantly lower than in the *N. hombergii* treatments, whereas in the 0.5 to 1 cm layer it was significantly higher than in all other macrobenthic treatments.
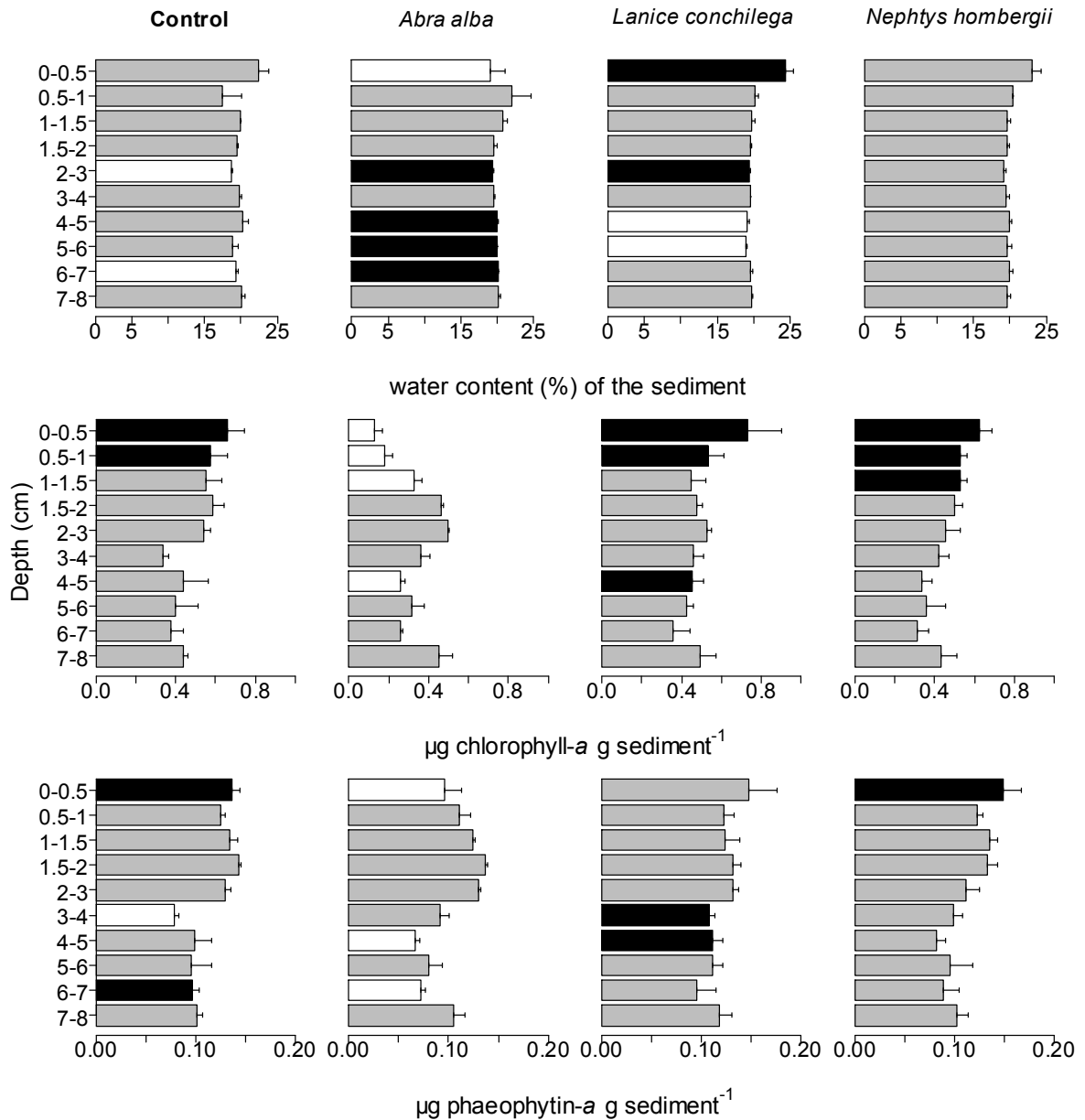

*Figure 2. Water content, chlorophyll-*a *and phaeophytin-*a *profiles in control,* Abra alba, Lanice conchilega, Nephtys hombergii *treatment (from left to right). Grey shades as in Figure 1. Error bars indicate SE*

A total of 80 nematode species was identified, with *Richtersia inaequalis*, *Sabatieria celtica*, *Sabatieria punctata*, *Microlaimus conothelis* and *Dichromadora cucullata* the 5 most abundant. Total species richness (S) and evenness (J') over the entire depth range did not differ among treatments, but Hill's indices N_1 and N_∞ were different (Appendix Table 4) and N_∞ was significantly higher in the *Nephtys hombergii* treatment than in the *Abra alba* (Tukey post-hoc p = 0.02) and control (Tukey post-hoc p = 0.04) treatments. This indicates that the commonest species (*i.e. Richtersia inaequalis*) was less dominant in the *N. hombergii* treatment. For N_1, no significant differences were detected with post-hoc tests. Evenness (J') depth profiles were similar in all treatments, whereas profiles of species richness (S) and Hill's diversity index N_1 differed significantly among treatments (Table 1, Figure 3). Species richness (S) was enhanced in the 0.5 to 1.5 and 2 to 3 cm layer of the *A. alba* treatment and in several depth layers between 1 and 7 cm depth in the *Lanice conchilega* treatment (Appendix Table 5). Similarly, Hill's diversity index N_1 was enhanced in several depth layers of the *L. conchilega* treatment (Figure 3, Appendix Table 5) and in the 2-3 cm layer of the *A. alba* treatment.

Nematode community

The vertical structure of the nematode community composition in the whole core was different among experimental treatments when analysed with raw data as well as with standardised, square root- transformed data (Appendix Table 6). Pairwise tests on raw data (whole core) showed a significant difference among treatments in nematode community composition in the 3-4 cm horizon between control and *Abra alba* treatment (t = 1.94; Monte Carlo p = 0.042). SIMPER analyses (Appendix Table 7) indicated that this difference could be accounted for by higher densities of *Sabatieria punctata* and *Spirinia* spp. at that depth in the *A. alba* treatment compared to control, while *Richtersia inaequalis* was completely absent in that horizon of the *A. alba* treatment. The analysis on the upper 2 cm only showed differences in vertical nematode community structure when analysed with raw data (Appendix Table 6).

Table 1. PERMANOVA results for the environmental variables, general univariate community variables and the 5 most abundant nematode species.

N: nematode abundance; S: species richness; J': evenness; ns: not significant. p-values obtained by permutation

| Variable	TR				Slice				TRxSlice			
	df	MS	Pseudo-F	p	df	MS	Pseudo-F	p	df	MS	Pseudo-F	p
Median grain size	3	29.75	2.83	ns	9	31.04	7.97	<0.001	27	6.18	1.59	ns
% Silt	3	29.30	1.38	ns	9	35.37	9.37	0.001	27	6.00	1.59	ns
Water content	3	1.28	2.37	ns	9	79.382	8.82	<0.001	27	3.17	2.18	0.005
Chl-a	3	59.78	1.75	ns	9	95.69	8.56	<0.001	27	18.15	1.62	0.001
Phaeophytin-a	3	0.00	1.02	ns	9	0.01	20.53	<0.001	27	0.00	1.94	0.014
Phaeophytin-a-like pigment	3	0.02	1.28	ns	9	0.01	13.26	0.001	27	0.00	1.21	ns
N	3	1133.40	4.10	ns	9	19825	54.54	<0.001	27	1566.70	4.31	<0.001
S (whole core, cm scale)	3	92.40	13.44	0.003	7	464.92	49.28	<0.001	21	19.43	2.06	0.017
S (upper 2 cm, 0.5 cm scale)	3	33.5	8.46	0.009	3	211.33	16.63	<0.001	9	34.72	2.73	0.020
J' (whole core, cm scale)	3	0.09	1.31	ns	7	0.04	1.23	ns	21	0.02	0.69	ns
Hill's N_1 (whole core, cm scale)	3	37.98	23.35	0.006	7	81.38	18.50	<0.001	21	9.66	2.20	0.011
N_1 (upper 2 cm, 0.5 cm scale)	3	22.04	17.67	<0.001	3	19.406	4.19	0.016	9	10.98	2.37	0.042
Hill's N_∞ (whole core, cm scale)	3	1.84	4.32	ns	7	2.67	3.02	0.009	21	1.45	1.64	ns
N_∞ (upper 2 cm, 0.5 cm scale)	3	4.52	15.66	0.005	3	1.92	1.90	ns	9	1.44	1.43	ns
Richtersia inaequalis	3	87.37	1.47	ns	9	2343.70	36.83	<0.001	27	139.58	2.19	0.006
Sabatieria celtica	3	53.54	3.29	ns	9	85.27	5.56	<0.001	27	30.77	2.01	0.013
Sabatieria punctata	3	18.41	0.89	ns	9	13.94	1.53	ns	27	11.66	1.28	ns
Dichromadora cucullata	3	9.43	4.09	ns	9	462.45	74.62	<0.001	27	40.13	6.47	<0.001
Microlaimus conothelis	3	20.30	0.80	ns	9	58.78	4.72	0.001	27	32.35	2.60	0.004
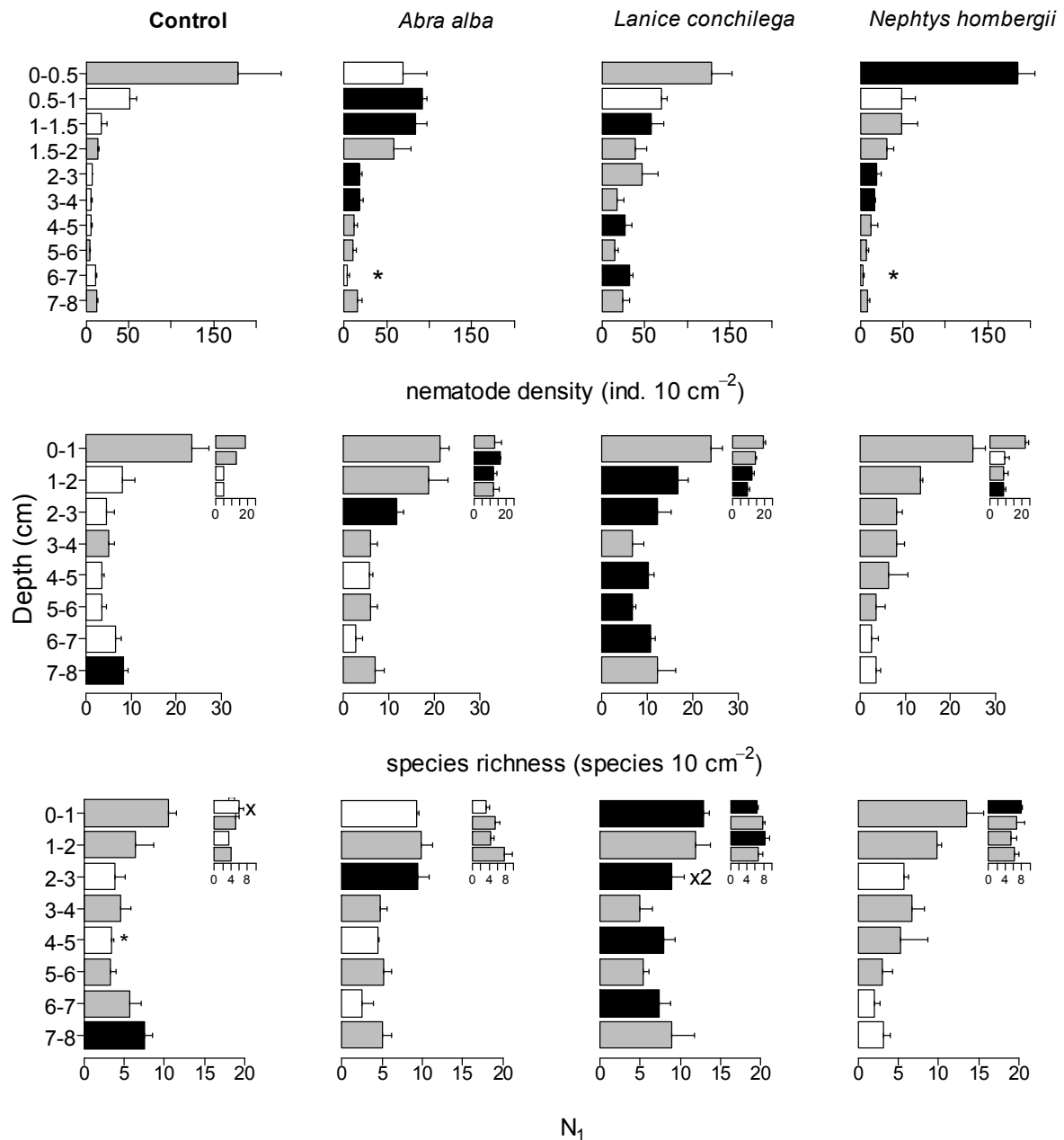
Figure 3. Nematode density and diversity (Species richness and Hill's diversity index N_1) profiles in control, Abra alba, Lanice conchilega, Nephtys hombergii *treatment (from left to right). Main graphs indicate analyses on whole core (cm scale); inset graphs show analyses on the upper 2 cm (0.5 cm scale). 'x': N_1 of the control 0-0.5 cm slice was only lower than the 0-0.5 cm slice of* N. hombergii. *"x2": N_1 of the* Lanice conchilega *2-3 cm layer was only higher than the N_1 of the control 2-3 cm layer. Grey shades as in Figure 1. Error bars indicate SE*

Pairwise tests on these raw data indicated a significant difference among treatments in community composition in the 0.5-1 cm horizon between the *A. alba* and *Nephtys hombergii* treatment (t = 1.80; Monte Carlo p = 0.049). SIMPER analyses (Appendix Table 7) showed that *R. inaequalis*, *Dichromadora cucullata* and to a lesser extent *Microlaimus conothelis* occurred in higher densities in the *A. alba* treatment of this horizon, while *Sabateria celtica* and *S. punctata* were more dominant in the *N. hombergii* treatment of this horizon.

Only the ANOSIM on the whole core based on raw data showed significantly different nematode community profiles among treatments (visualised in the second-stage MDS plot in Figure 4), which implies that the major nematode community changes take place over the entire depth range and are mainly a result of the response of the dominant species.
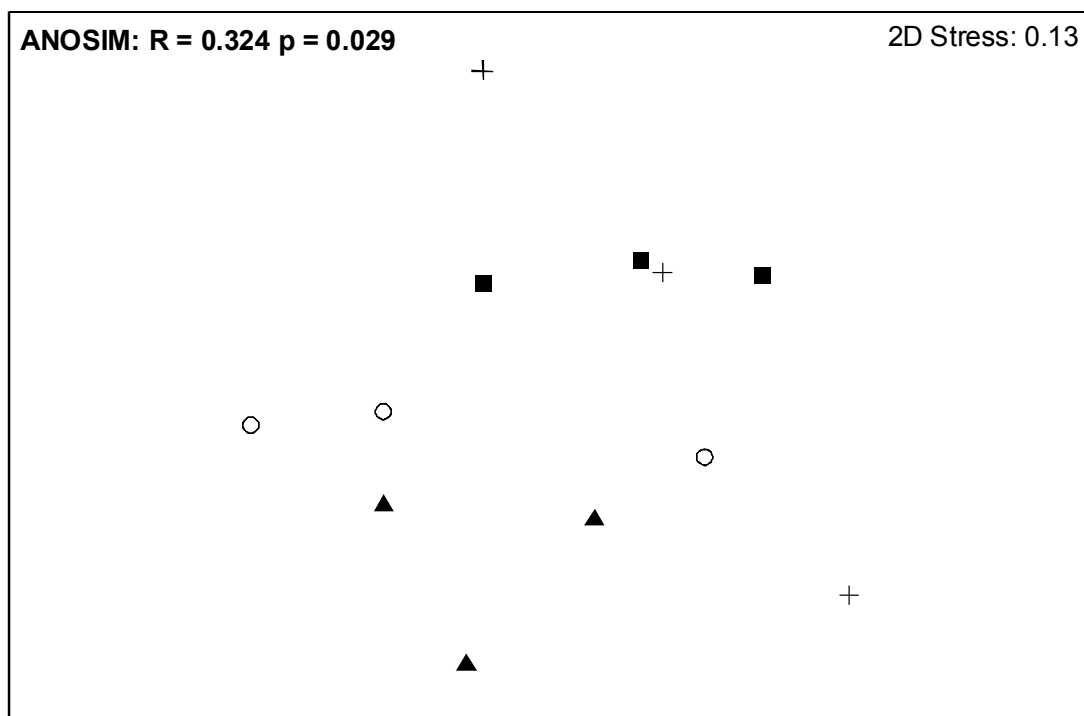
Figure 4. Second-stage MDS plot of the Spearman rank correlations between the nematode community profiles on whole core (cm scale and raw data) in control (■), Abra alba *(▲),* Lanice conchilega *(+) and* Nephtys hombergii *(○) treatments*

Dominant species

The vertical distribution of *Richtersia inaequalis* differed significantly among treatments (Table 1, Figure 5). The difference was mainly due to the significantly higher densities in the 0.5-2 cm horizons of the *Abra alba* treatment. Whereas the profiles of *Sabatieria punctata* were unaffected by the macrobenthic treatment, the profiles of its congener *Sabatieria celtica* (Table 1, Figure 5) differed significantly among treatments. *Microlaimus conothelis* (Figure 5) and *Dichromadora cucullata* (Figure 5) profiles also differed among macrobenthic treatments (Table 1) mainly due to higher densities in deeper layers of the *Lanice conchilega* (*S. celtica*, *M. conothelis*) and *A. alba* treatments (*S. celtica*), and differences in the surface layers (*D. cucullata, M. conothelis*).

Correlation between environmental variables and nematode distribution

The DISTLM routine with global BEST – analysis and BIC correction revealed that the combination of phaeophytin-*a* (9.15% of the variation explained) and water content (6.87% of the variation explained) explains the structure in the multivariate dataset (whole core, raw data) the best. However, this correlation is quite low ($R^2=0.138$). In contrast, the GLM model using combinations of environmental variables explains the variation in the univariate measures of the nematode community by between 17 and 50%, except for N_1 (2cm) and *Sabatieria punctata* (Table 2).

DISCUSSION

The present laboratory study demonstrates a significant effect of macrobenthos on the vertical distribution of nematodes, in terms of density, diversity and community composition, corroborating earlier observations on the structuring effect of macrobenthos on nematode communities (Ólafsson 2003; Pinto *et al.* 2006; Dashfield *et al.* 2008). However, by identifying nematodes to the lowest taxonomic level and resolving their vertical profile, we revealed that (1) these nematode community responses are due to increased densities of the dominant non-selective deposit feeding (*Richtersia inaequalis* and *Sabatieria celtica)* and epistrate feeding (*Dichromadora cucullata* and *Microlaimus conothelis*) species at certain depths, and that (2) the type of response depends on the macrobenthic functional traits (in terms of bioturbation and bio-irrigation). Furthermore, the densities of the nematode species most responsible for the

changes in community structure were related to the environmental variables altered by the resident macrofauna (*i.e.* water content and photopigments), suggesting ecosystem engineering effects of macrofauna on nematodes. Yet, these environmental variables did not fully explain the vertical structure of the nematode community composition (*cf.* the low R^2-value of DISTLM-analysis). (1) This may point at missing measurements of important structuring factors such as oxygen penetration depth (Steyaert *et al.* 2005a). (2) It may indicate a uniform response of the nematode species to the altered environmental variables, as reflected in the higher correlation with the univariate measures nematode density and diversity. (3) It demonstrates that apart from engineering the environmental variables, biotic interactions might play a role in the vertical structure of nematode community composition.

Table 2. Results of regression model, corrected for overdispersion, between nematode univariate measures and environmental variables that are significantly affected by macrobenthic presence. N: nematode abundance, S: species richness, N_1: Hill's diversity index

	Chlorophyll-a p-value	Phaeophytin-a p-value	Water content p-value	Adjusted D^2
N	< 0.001	< 0.001	< 0.001	0.50
S (whole core, cm scale)	0.023	< 0.001	< 0.001	0.39
S (upper 2 cm, 0.5 cm scale)	ns	ns	< 0.001	0.31
N_1 (whole core, cm scale)	ns	< 0.001	0.016	0.26
N_1 (upper 2 cm, 0.5 cm scale)	ns	ns	ns	ns
Richtersia inaequalis	< 0.001	< 0.001	ns	0.40
Sabatieria celtica	ns	< 0.001	ns	0.33
Sabatieria punctata	ns	ns	ns	ns
Dichromadora cucullata	0.007	< 0.001	ns	0.33
Microlaimus conothelis	0.015	< 0.001	ns	0.17

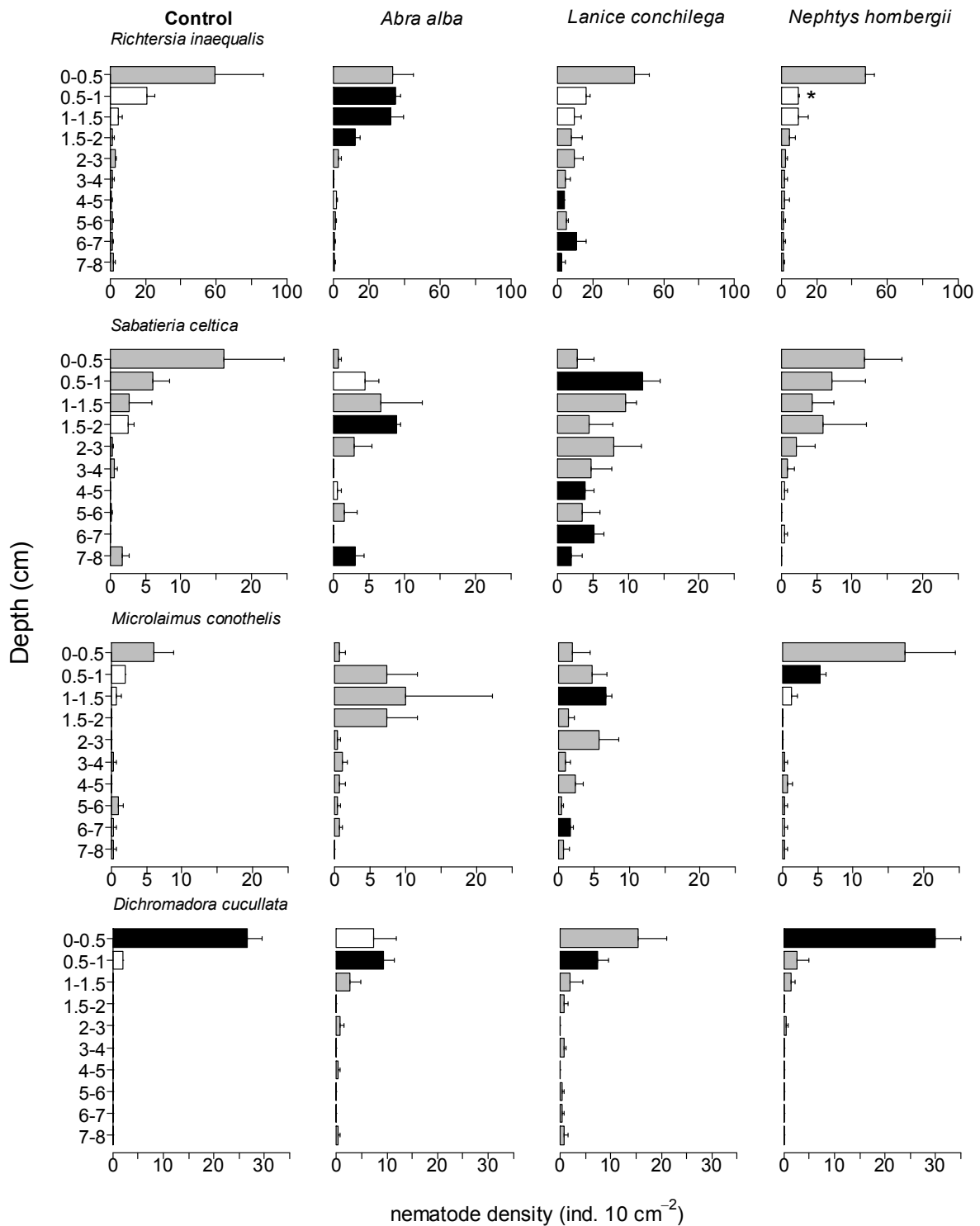*Figure 5.* Richtersia inaequalis, Sabatieria celtica, Microlaimus conothelis, Dichromadora cucullata *density profiles in control,* Abra alba, Lanice conchilega *and* Nephtys hombergii *treatments (from left to right) * Significantly lowest values. Grey shades as in Figure 1. Error bars indicate SE*

Nematodes experienced higher mortality in the control sediment without macrofauna, and those that survived concentrated in the upper centimetre of the sediment, apparently because bioturbation and bio-irrigation were lacking, which reduced subsurface oxygen concentrations and increased concentrations of toxic metabolites (Steyaert *et al.* 2003, 2005a, 2007; Franco *et al.* 2008c). In addition, nematode diversity in these controls was impoverished in several depth layers compared to the sediments with macrofauna present. Widdicombe *et al.* (2003) pointed out that bioturbation is essential for species diversity by producing heterogeneity or complexity within the sediment. Importantly, the significantly different treatment effects suggest differing underlying mechanisms (as hypothesised in the following subsections) for the observed nematode community responses depending on macrofaunal functional identity.

Abra alba: exploitative competition and disturbance at the surface vs. faecal pellets in subsurface

Nematode density was significantly lower in the surface layer reworked by *Abra alba*. Nematodes seemed to have migrated downwards, resulting in higher densities between 0.5 and 4 cm sediment depth, which was particularly clear for the dominant species *Richtersia inaequalis, Dichromadora cucullata* and *Sabatieria celtica*, as shown by the different community profile of the *A. alba* treatment based on raw data (whole core, response of dominant species not weighed down, Figure 4). Despite the substantial bioturbation activity of *A. alba* (Braeckman *et al.* 2010), its activity is probably limited to sediment reworking and does not consist of deep organic matter burial (Graf 1989; Levin *et al.* 1997). During the present experiment, most of the labile organic matter was consumed by *A. alba* (emptied chl-*a* profile in Figure 2) and little to none was left for subsurface transport. Hence, the downward migration of nematodes cannot be linked to transport of food from the surface to depth. It therefore presumably relates to the depletion of food resources (diatoms) of the non-selective deposit and epistrate feeders *sensu* Wieser (1953) due to deposit feeding activities of *A. alba* at the surface. In addition, the sediment sucking siphons may have caused a substantial physical disturbance in the first 0.5 cm, thereby chasing the nematodes downwards. Direct

predation seems unlikely as the size of particles taken up by *A. alba* siphons is smaller than the size of nematodes (Austen *et al.* 1998)[4]. The enhanced water content and thus related interstitial space around the bivalve might have facilitated the mobility of nematodes (McIntyre 1969). Furthermore, the downward shift of *R. inaequalis* and *D. cucullata*, both known to be intolerant to oxygen stress (Steyaert *et al.* 2007; Franco *et al.* 2008c) suggests the presence of small oxygen pulses into the sediment in close vicinity of the bivalve, likely facilitating the deeper survival of these species (Bouchet *et al.* 2009). Reise (1983) related an increased turbellarian density in close vicinity of the deposit feeding bivalve *Macoma balthica* to the subsurface creation of micro-oxic zones and nutrient-stimulated micro-organism growth. Similarly, in the present experiment, the downward shift of the nematodes was concentrated between 0.5 and 3 cm, which lies exactly in the working area of *A. alba*, where it creates feeding pits (Maire *et al.* 2007) and deposits faecal pellets (Amouroux *et al.* 1989), both potentially sustaining bacterial populations (Solan and Wigham 2005).

Nephtys hombergii – no effect

Our data indicate no engineering effects of *Nephtys hombergii* on nematodes. Apart from a lower dominance of species (N$_\infty$ in Table 1 and Appendix Table 4), the vertical structure of the nematode community composition was not different from the pattern found in the control sediments. Further, the total nematode density was not affected, which excludes predation of this polychaete on the nematodes (Tita *et al.* 2000). Moreover, *N. hombergii* did not alter the environmental variables measured and does not introduce oxygen into the sediment (Braeckman *et al.* 2010), hereby precluding engineering effects on nematodes via modification of sediment characteristics.

Lanice conchilega: deep sediment oxygenation

The higher survival of nematodes and the redistribution of several dominant species in the *Lanice conchilega* treatment suggest that this polychaete has engineering capacities that facilitate nematodes to dwell in deeper layers (Tita *et al.* 2000, Pinto *et al.* 2006). In

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[4] Nevertheless, it is possible that the small juvenile nematodes were preyed upon. However, the proportion of juveniles was equal in all treatments, which precludes this hypothesis.

contrast to *Abra alba*, *L. conchilega* did not deplete the chl-*a* and phaeophytin-*a* repository at the surface (and even slightly enhanced chl-*a* and phaeophytin-*a* concentrations at depth when compared to the *A. alba* treatment) (Figure 2). In addition, regular and deep oxygen pulses along the tube walls (Forster and Graf 1995) may be beneficial to nematode survival as well. The recorded high nematode density at depth was mainly visible in *Sabatieria celtica* and to a lesser extent in *Microlaimus conothelis* and *Richtersia inaequalis* profiles. Whereas it is generally accepted that *S. punctata* and *S. pulchra* are resistant (Jensen 1981, 1983; Steyaert *et al.* 2005a) to anoxia and associated elevated hydrogen sulphide concentrations, observations on the distribution of *S. celtica* on the one hand and *M. conothelis* on the other are less clear: Soetaert *et al.* (1994, 1995) show a deep and opportunistic distribution of *S. celtica*, whereas Jensen (1983) and the control treatment in this study point at a preference for oxygenated layers. Similarly, Heip *et al.* (1985), Wetzel *et al.* (1995) and Steyaert *et al.* (1999) point towards a deep distribution of *M. conothelis*, whereas the control treatment in the present study showed avoidance of deep layers. Consequently, the presence of the long and slender nematode species *S. celtica* and *M. conothelis* in the deep layers of the *L. conchilega* treatment might be linked to the regular oxygen pulses that reach this depth while at the same time these species can tolerate anaerobic non-irrigated intervals (Reise and Ax 1979). The presence at depth of the stout species *R. inaequalis*, which is probably vulnerable to oxygen diffusion limitations due to its large body width (Wetzel *et al.* 1995; Soetaert *et al.* 2002) further illustrates the *L. conchilega*-induced oxygenation of deeper sediment layers. In addition to the oxygenation of deep sediment layers, deposit feeding nematodes may profit from the stimulation of bacterial populations in the mucuous linings of the tube (Ziegelmeier 1952) or/and in the sediment surrounding the tubes (Solan and Wigham 2005).

CONCLUSIONS

The control treatment was detrimental to nematode survival compared to the *Lanice conchilega* treatment, most probably due to compaction of the sediment, lack of irrigation and associated shallower oxygen penetration depth in the absence of bioturbating macrofauna. In contrast, *Abra alba* and *L. conchilega* showed ecosystem engineering capacities that extended the possible habitat area to a depth of respectively
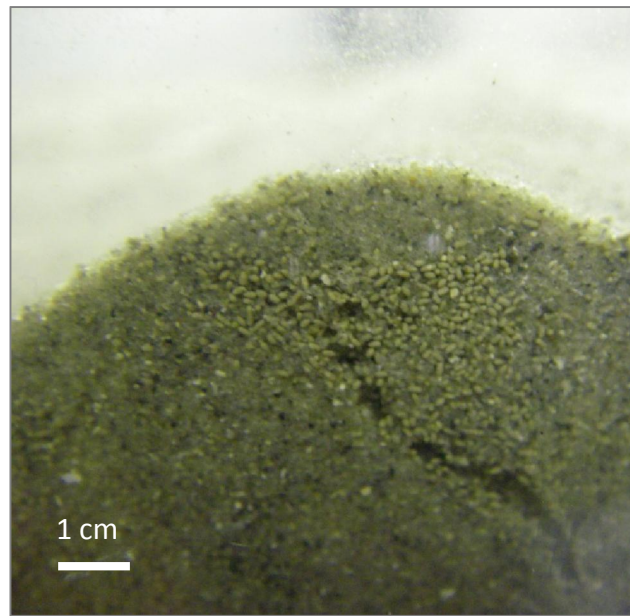
3 and 7 cm probably through removal of sulphide and ammonia and possibly also by stimulation of bacteria as food sources (respectively in faecal pellets and along tube walls). This indicates again that ecosystem engineering macrobenthos is essential for the survival of lower parts of the food web, such as foraminifera (Bouchet *et al.* 2009) and nematodes (Van Colen *et al.* 2009b). It is important to note, however, that the outcome of our experiment is likely strongly habitat-dependent and the results presented should therefore strictly be interpreted in terms of subtidal fine sandy habitats under a regular hydrodynamic regime. For instance, muddy sediments are subjected to slow diffusive pore water transport while advective transport dominates in coarse sediments. Organisms in the first sediment type will therefore benefit from additional macrobenthic engineering of pore water flows, while physical forces may overrule macrobenthic engineering effects in sandy sediments (Ólafsson 2003; Meysman *et al.* 2006a). Furthermore, depletion of food resources may be less prevalent in biofilm covered tidal flat sediments (Kennedy 1993) or during the seasonal mass input of phytodetritus in subtidal sediments (Franco *et al.* 2010). Overall, our results reveal that functionally contrasting macrobenthic identities shape nematode communities in different ways. Such different engineering effects may maintain the role of nematodes in ecosystem functioning (Yeates *et al.* 2009) and the present study therefore highlights the need for the conservation of macrobenthic functional diversity.
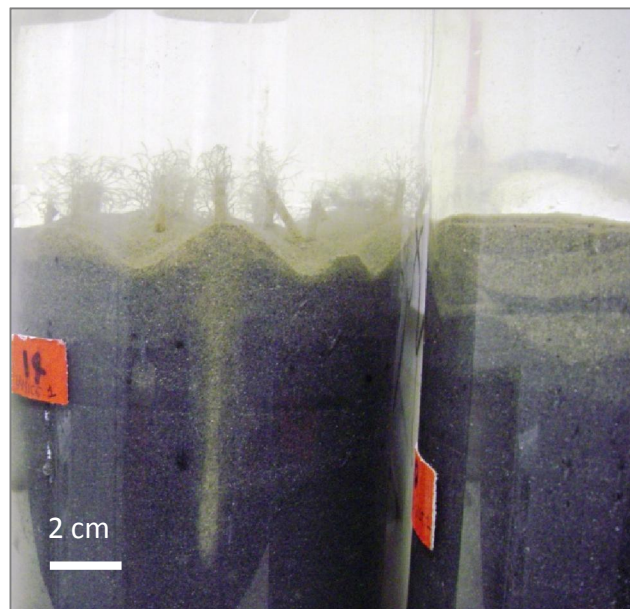
ACKNOWLEDGEMENTS

Abra alba: subsurface depositions of faecal pellets

Oxygenated sediment around *Lanice conchilega* tube (left) and
mixed surface layer of the physical mixing treatment (right)