

## The effect of the dominant polychaete *Scolelepis squamata* on nematode colonisation in sandy beach sediments: An experimental approach

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### ABSTRACT

The effect of an abundant sandy beach polychaete, *Scolelepis squamata*, on the colonisation of defaunated sediments by marine nematodes indicates that sandy beach fauna can be partially controlled by biological interactions within and across size groups. Experimental cores, equipped with windows allowing infaunal colonisation, were filled with defaunated sandy beach sediment containing two different treatments with and without *S. squamata*. These cores were inserted into microcosms filled with sediment with indigenous meiofauna collected from the field. The treatments were incubated in the laboratory at ambient temperature and salinity for 2, 7, 14 and 21 days, in order to follow the colonisation process of the defaunated sediments by the indigenous nematode fauna over time. Nematodes initially colonised both treatments, with abundances of up to 10% of the densities in the control; after 2 weeks, nematode densities in the cores without *S. squamata* surpassed the control densities. Nematode assemblages in both treatments were not species rich, and also differed in composition from the natural assemblages. The most successful colonising species, *Enoplolaimus litoralis*, was rare in the surrounding sediment, suggesting that colonisation was determined by species-specific characteristics such as body size, motility and feeding strategy. Initially the presence of macrofauna did not affect the nematode community composition, but after 2 weeks of the experiment, the presence of the polychaete seemed to facilitate the earlier establishment of non-opportunistic species.

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### 1. Introduction

Colonisation experiments are widely used to evaluate the effects of disturbance on benthic community structure (Alongi et al., 1983; Decker and Fleeger, 1984; Colangelo et al., 1996; Zhou, 2001). Some studies have reported the ability of meiofauna, more particularly nematodes, to colonise sediments via lateral migration (Chandler and Fleeger, 1983; Schratzberger et al., 2004; Gallucci et al., 2008). They suggested that dispersal through the water column is responsible for the colonisation of large-scale defaunated areas, whereas small-scale colonisation events are determined by active infaunal migration. Because of the strong hydrodynamic forces on sandy beaches, both processes (passive migration through the water column and active infaunal migration) may be important in establishing the nematode assemblages during a colonisation event, but at different spatial scales. Colonisation in sandy beaches is an important ecological process, and is investigated here to

understand how diversity and community structure of nematodes, a dominant sandy beach group, re-establish after a disturbance event. Natural disturbances occur daily in sandy beaches. They are driven by wave-action, which erode and deposit part of the intertidal sediments, or they can be caused by human-activity, such as the recreational use of the intertidal areas, or beach nourishment which is widely applied coastal defence technique along the coast of North America and Europe (Speybroeck et al., 2006). Beach re-colonisation processes occur after both small- and large-scale defaunating events. This is a successional process, and different organisms may not have the same ability to colonise the newly available niches (Horn, 1981).

Most experimental studies have investigated the colonisation of defaunated sediments under the absence of macrofauna (Sun and Fleeger, 1994; Zhou, 2001; Schratzberger et al., 2004; Gallucci et al., 2008). Macrofaunal organisms influence meiofaunal colonisation directly through competition, predation or bioturbation (Sandnes et al., 2000), or indirectly through changing the physical and chemical properties of the sediment by their activity (Rosenberg et al., 2001; Van Colen et al., 2009). In addition, the tidal regime of sandy beaches might also influence nematode

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colonisation processes, since sandy beaches are essentially physically-driven ecosystems (Defeo and McLachlan, 2005). Although it is assumed that biological interactions may be more influential in structuring small-scale benthic communities in dissipative beaches than in reflective ones (Defeo and McLachlan, 2005), the importance of biotic interactions versus physical drivers in sandy beaches is still unclear (Schlacher et al., 2008).

*Scolecipis squamata* is the dominant polychaete in the upper to middle intertidal sediments of De Panne beach (Belgian coast, North Sea). This species is a cosmopolitan polychaete significantly abundant in the macrobenthos from sandy beaches of North and South Atlantic, North Pacific, Indian Ocean and Mediterranean Sea (Souza and Borzone, 2000). It is a deposit-feeding species and reaches abundances higher than 100 ind. m<sup>-2</sup> in the studied beach (Degraer et al., 2003) and is known to be one of the few species least affected by beach nourishment and rapidly recovering their densities after disturbance (Peterson et al., 2006). Through its burrowing and deposit-feeding activity and by producing pseudofaeces, this macrobenthic organism can modify the environment and, as shown for many other polychaete species, this species is also expected to interact negatively or positively with nematodes through its overall activities in the sediment (see Ólafsson, 2003 for a review; Braeckman et al., 2010). Furthermore, the occasional predation by *S. squamata* on larvae and juveniles of other macrobenthic species and on meiobenthic harpacticoid copepods and ostracods (Dauer, 1983) may also affect nematodes although they were so far not recorded as a food source for *S. squamata* (Scholz et al., 1991).

The aim of the present study was to investigate the process of colonisation of sandy beach sediments by nematode assemblages in the presence or absence of *Scolecipis squamata*. A laboratory microcosm experiment was set up to test two hypotheses. Despite some limitations, this microcosm experiment can unravel biological interactions between a dominant polychaete and nematodes, and the results can provide an initial understanding of how interactions occur in nature. Our first model states that colonisation is a species-specific successional process, i. e. some nematodes are more capable than others of colonising a newly available environment based on the body-size dependency of nematode communities in colonising defaunated patches (Schratzberger et al., 2004; Gallucci et al., 2008). We stated a null hypothesis that the nematode communities do not change between sampling dates; the alternative hypothesis is that there is a directional change in the nematode community. Our second model asserts that the dominant polychaete *S. squamata* can facilitate the nematode colonisation by its overall activities. Here, we test the null hypothesis that the nematode community in newly colonised sediments is unaffected by the presence of *S. squamata*, and the alternative hypothesis is that *S. squamata* affects the nematode colonisation.

## 2. Material and methods

### 2.1. Study area and sediment sampling

Sediment from the upper 10 cm was collected in the upper intertidal level of the ultradissipative sandy beach of De Panne (51°05'30"N, 02°34'01"E) in front of the nature reserve "Westhoek reservaat", four weeks prior to the experimental set up (8 October 2007). The intertidal area is approximately 440 m wide and has four runnels parallel to the water line. The sampling area was restricted to sandbars in order to avoid areas with a different community composition, since runnels and sandbars are assumed to have dissimilar nematode communities (Gingold et al., 2010). The mean spring and neap tide ranges are 5 m and 3 m, respectively; modal breaker height is 0.5 m and modal wave period is 3 s (Department of Waterways and Coast, unpublished data). The beach slope is about 1:90 to 1:100 (Gheskiere et al., 2004). In the sampling area, *Scolecipis squamata* reaches maximum densities of 700 ind. m<sup>-2</sup> in summer and 1100 ind. m<sup>-2</sup> in winter (Degraer et al., 1999).

### 2.2. Experimental set-up

Sediment previously collected was defaunated and the organic-matter removed by burning to 500 °C for 4 h, using a muffle furnace (Zhou, 2001). One day before the experimental set up (5 November 2007), triplicate field control (FC) samples were collected by means of Perspex cores (10 cm<sup>2</sup>) to a depth of 10 cm, in order to collect baseline information on the resident nematode community, from the same sandbar area that was visited on October 8. Large volumes of sand were also collected from the beach surface (to a depth of 10 cm). This sediment was homogenised in the field. Individuals of *Scolecipis squamata* were collected by sieving sediment from the upper intertidal level of the beach, where it is the most abundant macrofaunal organism (Degraer et al., 1999). Until the experiment was set up, the polychaetes were kept in an aquarium filled with sand and oxygenated seawater.

Six microcosms, each consisting of a plastic aquarium (72 l) were filled with homogenised sediment inhabited by natural meiofaunal and macrofaunal sandy beach communities, to a depth of 12 cm. The microcosms were left untouched for one day to allow the community to stabilise. One corner of the aquarium (96 cm<sup>2</sup>) was reserved for the placement of a water pump, silicone tubes and air stones in an individual plastic container, avoiding any disturbance of the sediment. Thirty-six experimental cores (10 cm<sup>2</sup>) were allocated to three types of treatment (see below) and were randomly pushed into the sediment of different aquaria at a fixed distance of 10 cm (Fig. 1A). Before the experimental cores were added (pushed in the sediment), a similarly sized core sample of sediment was removed

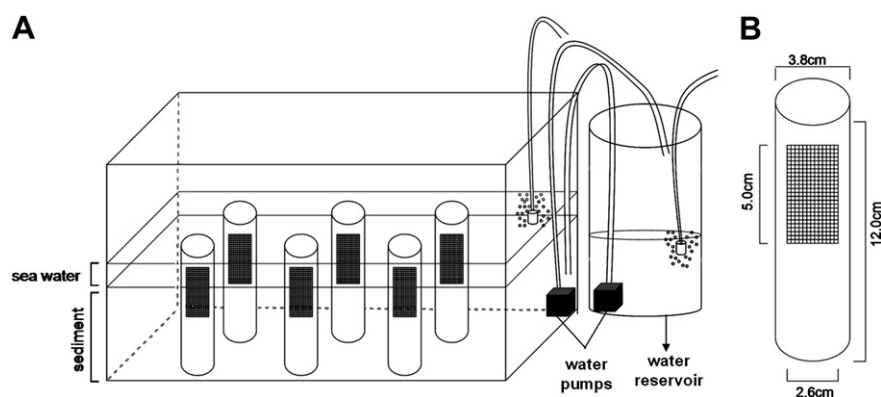


Fig. 1. Schematic drawing of experimental set up: A: aerated microcosms (57 × 37 × 31 cm), B: syringes with windows of 5 cm × 2.6 cm filled with different kind of treatments.

from the aquarium, in order to reduce disturbance when introducing the experimental cores. The experimental cores had two opposite lateral windows, each measuring 5.3 cm × 2.6 cm and covered by gauze with a mesh size of 1 mm (Fig. 1B). These lateral windows allowed the meiofauna to migrate from the adjacent sediment. The upper 2 cm of the gauze was in contact with the water column, in order to allow mimicking of tides inside these cores, but the edge of the cores was never surpassed by the water column.

Three types of treatments in the experimental cores, distributed randomly among the 6 aquaria, were:

- (1) Indigenous control (IC): natural sediment with indigenous community, collected simultaneously with the sediment that was used to fill the aquarium. This type of control was used to check for a possible enclosure effect caused by the use of the core;
- (2) Azoic treatment (AT): defaunated sediment;
- (3) *Scolecopsis* treatment (ST): defaunated sediment + 1 specimen of *Scolecopsis squamata*, which corresponded to its natural density.

Immediately after the experiment was set up, the sediment in the aquarium was covered with 3.5 cm filtered seawater of natural salinity (30). The experiment ran in a temperature-controlled room at a day and night light regime of 12:12 h and a constant temperature of 15 °C. Tides were simulated twice a day, forming a water column of 3.5 cm above the sediment layer. The water entered the experimental cores through the upper 2 cm of gauze, covering the lateral windows of the core. Sediments were submerged for 2 h and exposed to the air for 10 h. Changes in salinity of the seawater were monitored daily, and increases due to evaporation were avoided by adding deionised water to the aquaria, thereby maintaining the natural salinity of 30. Three replicates of each treatment were removed from different aquaria and transferred to a plastic container at 2, 7, 14 and 21 days post-placement, during the period of simulated low tide. At the same time, control samples (AQ) were randomly collected from the aquarium sediment, with a 10 cm<sup>2</sup> core. Immediately after the removal of the experimental cores, the holes were filled with other cores of the same size, to prevent the surrounding sediment from collapsing. All samples were preserved in a 10% formaldehyde solution until sample processing.

### 2.3. Sample processing in the laboratory

Nematodes were extracted from the sediment by centrifugation with Ludox (Heip et al., 1985). Macrofauna was excluded by means of a 1-mm sieve. All organisms retained on a 38-µm sieve were stained with Rose Bengal, identified and counted under a dissecting microscope. A random subsample of 100 nematodes was transferred to De Grisse solution (De Grisse, 1969) and mounted on slides for further identification to genus and species. Cores from the *Scolecopsis* treatment were checked to assess if the *Scolecopsis* specimens were still alive on the day of sampling. If this was not the case, the sample was excluded from the analyses.

### 2.4. Data analyses

Nematode assemblages from all the treatments and sampling dates were analysed by means of univariate and multivariate techniques. Total densities per 10 cm<sup>2</sup>, richness (*S*), and diversity (Shannon diversity index –  $H' \log_e$ ) were calculated for each treatment. The differences between nematode densities of FC and AQ, and between FC and IC were analysed by *t*-tests. Differences in nematode densities among sampling times in AQ and IC were analysed by one-way ANOVA, after checking for assumptions.

The experimental effects on total nematode density per 10 cm<sup>2</sup>, densities of the successful colonising nematode species, species richness (*S*), and diversity ( $H'$ ) were tested by two-way analysis of variance (two-way ANOVA). When significant differences were detected, Tukey HSD tests for unequal *N* were applied to test for pairwise differences, since the design was unbalanced due to the death of the polychaete in one of the replicates of day 7. Data were  $\log(x + 1)$  transformed in order to meet the assumption of homogeneity (Cochran's test). Differences in nematode community structure were analysed by non-metric Multi-Dimensional Scaling (MDS) using the Bray–Curtis Similarity on fourth-root transformed data for each sample. A one-way PERMANOVA was performed to analyse differences in the community structure among FC, AQ and IC whereas a two-way design was applied to check for differences in the community structure among treatments (AQ, IC, AT and ST) and times over the course of the experiment (Anderson et al., 2008). Since a PERMANOVA test can show significant differences between groups, but does not distinguish between a difference due to factor effects or dispersion (variance), the homogeneity of multivariate dispersion was tested with PERMDISP, using distances among centroids calculated in treatment × time group. The PERMDISP test was never significant, indicating equally dispersed distances to centroids. In the case of a significant result in the PERMANOVA design, pairwise tests for the significant term were performed. In cases of a restricted number of possible permutations in pairwise tests, *p*-values were obtained from Monte Carlo samplings (Anderson and Robinson, 2003). The species contributing most to within-group similarity were identified by the two-way crossed SIMPER analysis. All the multivariate analyses and the calculations of *S* and  $H'$  were performed using the PRIMER v6 with PERMANOVA + add-on software package (Anderson et al., 2008); the *t*-test and ANOVA were done using STATISTICA 7.0.

## 3. Results

### 3.1. Univariate measurements

The densities recorded in AQ samples were significantly different from the values recorded from the field samples (time zero) (Fig. 2; *t*-test,  $t = 4.52$ ,  $p = 0.01$ ) and corresponded to approximately 50% of the *in situ* values. The densities in the aquarium controls appeared to change significantly over the course of the experiment (Fig. 3; one-way ANOVA,  $F_{3,8} = 4.21$ ,  $p = 0.04$ ), but a post-hoc test did not show differences in aquarium densities between the different incubation times (Tukey HSD, all  $p > 0.05$ ). In the case of the indigenous controls, the density also corresponded to less than 50% of the values recorded from the field samples (Fig. 2; *t*-test,  $t = 4.52$ ,  $p = 0.01$ ). There was no significant change

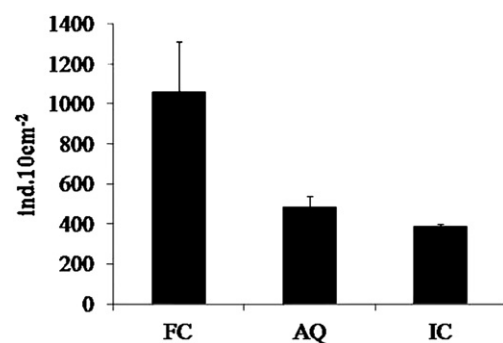
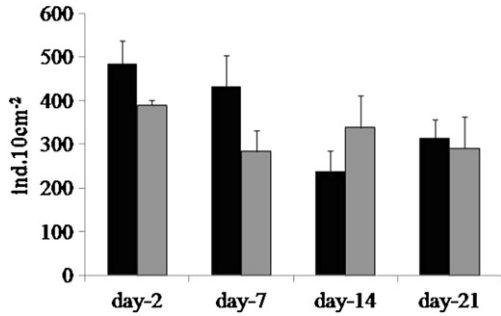


Fig. 2. Mean total nematode density in the field control ( $t = 0$ ) and in the initial stages of the experiments ( $t = 2$ ). FC: field control, AQ: aquarium control, IC: indigenous control. Error bar represents  $\pm$ SE ( $n = 3$ ).



**Fig. 3.** Mean total nematode density in the controls over the 21 days of the experiment. Black bars represent AQ (aquarium control) and grey bars represent IC (indigenous control). Error bar shows  $\pm$ SE ( $n = 3$ ).

over time in the densities of IC over the course of the experiment (Fig. 3; one-way ANOVA,  $F_{3,8} = 0.75$ ,  $p = 0.55$ ).

Since the nematode species *Enoplolaimus litoralis* was dominant in both AT and ST treatments after 1 week of incubation, it was further analysed separately. Fig. 4A and B represent total nematode densities and *E. litoralis* densities, respectively, in all treatments over time. It illustrates that besides the high densities of this species in the treatments AT and ST, it was only sporadically present in the control treatments (AQ and IC). Nematode densities, the diversity index  $H'$ , and *E. litoralis* densities were significantly affected by the time  $\times$  treatment interaction in a two-way ANOVA (Table 1, Fig. 4). Tukey HSD for unequal N (Table 1) indicated that there was no difference between AQ controls and IC, and no difference in the nematode densities between AT and ST. Total nematode densities were initially higher in AQ samples compared to ST and AT samples; however, this pattern was significantly reversed from 2 (AT) or 3 (ST) weeks onwards (Fig. 4A). From 7 days onwards,  $H'$  values in AQ cores were always significantly higher than in AT and ST (Fig. 4D). However, at day 14 a significant difference was recorded for diversity  $H'$  and *E. litoralis* density

between the defaunated treatments with (ST) and without the polychaete (AT), with  $H'$  being higher in ST compared to AT and *E. litoralis* less abundant in ST (Table 1).

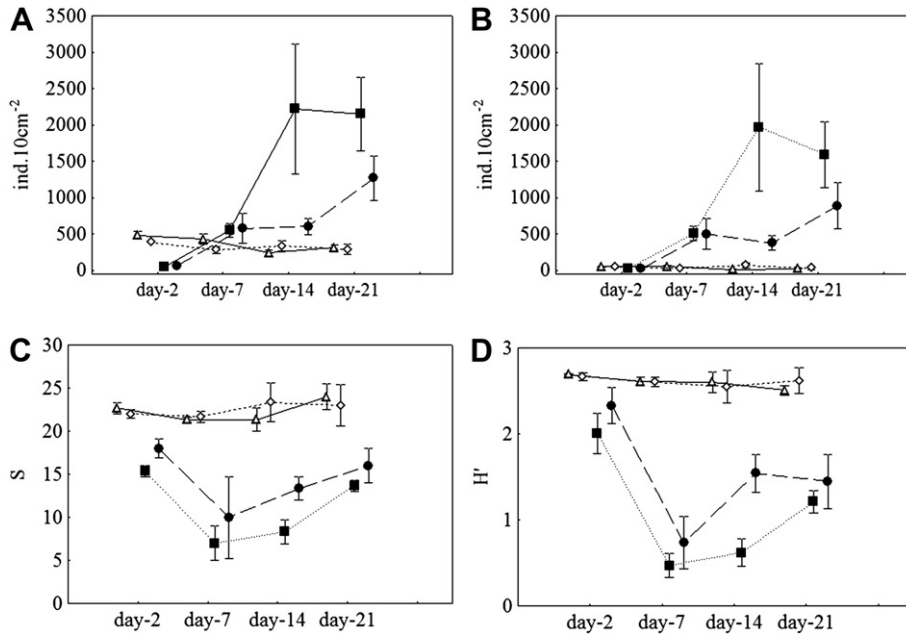
Species richness ( $S$ ) was not significantly affected by the time  $\times$  treatment interaction. However, significant differences were observed for both treatment and time separately (Table 1, Fig. 4C). Tukey HSD for unequal N indicated that there was no difference between AQ control and IC, and between AT and ST and day-2 was significantly different of day-7.

### 3.2. Community structure

A mean of 11.284 individuals were found in this study, belong to 53 species and 4 unidentified genera (Appendix). No significant differences in nematode community composition were observed between FC, AQ, and IC treatments at the beginning of the experiment (one-way PERMANOVA: Pseudo- $F_{2,8} = 1.65$ ,  $p = 0.056$ ). In addition, nematode communities from both AQ and IC changed significantly over time (one-way PERMANOVA; Pseudo- $F_{3,8} = 1.996$ ,  $p = 0.004$  and Pseudo- $F_{3,8} = 1.48$ ,  $p = 0.03$ , respectively) (Fig. 5), although pairwise comparisons were not able to detect at which time of the experiment this difference occurred (Table 2B).

The nematode community was significantly affected by the time  $\times$  treatment interaction in two-way PERMANOVA (Table 2A). The pairwise test showed that the nematode communities from AQ and IC were significantly different from the communities encountered in AT and ST treatments at day 2. On the following sampling days, nematode communities from AQ differed significantly only from those in the AT treatment (Fig. 4, Table 2B). There were no significant differences in nematode communities from the AT and ST treatments.

*Sigmaphoranema rufum* was the dominant species in the AQ and IC treatments, with mean densities ranging from 54 to 109 ind. 10 cm<sup>-2</sup> and 36 to 62 ind. 10 cm<sup>-2</sup>, respectively. In AT and ST, *Enoplolaimus litoralis* was the dominant species, with 22–1967 ind. 10 cm<sup>-2</sup> in AT and 21–887 ind. 10 cm<sup>-2</sup> in ST (Appendix). These two species showed a mean density of 207 and 152 ind. 10 cm<sup>-2</sup>, respectively, in the field



**Fig. 4.** Univariate indices for nematode assemblages over the 21 days of the experiment. Treatment results (black symbols) were plotted against data obtained for the aquarium controls (open symbols) that served as potential species pool for colonisation of the defaunated sediment. A: mean total nematode density, B: *E. litoralis* density, C: species richness ( $S$ ), D: Shannon diversity index ( $H'$ ). Error bar represents  $\pm$ SE ( $n = 3$ ; except day 7 for ST,  $n = 2$ ). AQ: triangles, IC: diamonds, AT: squares, ST: circles.

**Table 1**  
Results from two-way ANOVA for the treatment and time effects on nematodes univariate measurements (Field controls were omitted in the analysis), and Tukey HSD unequal N for nematode total density, Shannon diversity and *S. squamata* density.

Two-way ANOVA	Treatment		Time		Treatment × time	
	$F_{(3,31)}$	<i>p</i>	$F_{(3,31)}$	<i>p</i>	$F_{(9,31)}$	<i>p</i>
Density	4.95	0.006	32.06	<0.001	19.96	<0.001
Richness ( <i>S</i> )	49.58	<0.001	6.60	<0.001	1.48	0.201
Diversity ( <i>H'</i> )	78.65	<0.001	15.17	<0.001	4.58	<0.001
<i>E. littoralis</i> density	39.61	<0.01	14.52	<0.01	10.20	<0.01
Pairs compared	Density		<i>H'</i>		<i>E. littoralis</i> density	
AQ × IC	n.d.		n.d.		n.d.	
AQ × AT	Day-2 (AQ > AT), days 14–21 (AQ < AT)		Days 7–14–21 (AQ > AT)		Days 7–14–21 (AQ < AT)	
AQ × ST	Day-2 (AQ > ST), day-21 (AQ < ST)		Days 7–14–21 (AQ > ST)		Days 7–14–21 (AQ < ST)	
IC × AT	Day-2 (IC > AT), days 14–21 (IC < AT)		Days 7–14–21 (IC > AT)		Days 7–14–21 (IC < ST)	
IC × ST	Day-2 (IC > AT), days 21 (IC < AT)		Days 7–14–21 (IC > ST)		Days 7–14–21 (IC < ST)	
AT × ST	n.d.		Day 14 (AT < ST)		Day 14 (AT > ST)	

AQ: aquarium control, IC: indigenous control, AT: azoic treatment, ST: *Scolecopsis* treatment, n.d.: no difference.

control. High densities of particular species, such as *Daptonema normandicus* (63 ind. 10 cm<sup>-2</sup>) and *Metadesmolaimus* sp.1 (24 ind. 10 cm<sup>-2</sup>) at day 14, were found in the presence of *Scolecopsis* (ST); both species only reached high comparable densities at day 21 in the treatment without the polychaete (AT) (Appendix).

The species contributing to the similarity within each treatment indicated by two-way crossed SIMPER are listed in Table 3. Within-group similarity in AQ and IC was mainly determined by *Sigmo-phoranema rufum*, whereas *Enoplolaimus littoralis* was much more important in the AT and ST (Table 3).

## 4. Discussion

### 4.1. Experimental set-up

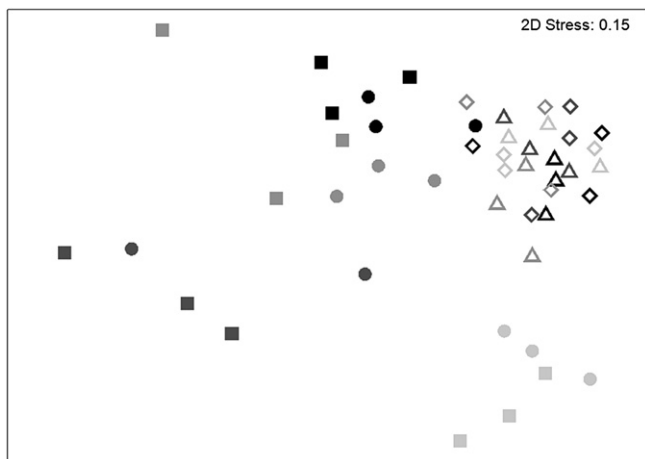
The manipulation of the sediment (sampling, homogenisation, transport to the laboratory and setting up of the microcosms) clearly influenced the nematode communities in terms of density, but not in terms of community structure since most of the species recorded from the field were also found in the AQ samples during the entire duration of the experiment. The slight reduction of nematode densities over the course of the experiment in the AQ samples may be due to the colonisation of the cores, as the timing of this decrease

coincided with the increase in densities in the colonising cores. Indeed, this relatively small temporal change in AQ was not detected by the post-hoc tests. Significant changes over time in control nematode assemblages are observed in small microcosms (such as beakers of about 1 l) as well (Schratzberger and Warwick, 1999; Schratzberger et al., 2004). Considering that nematodes are not able to actively migrate long distances (Sherman and Coull, 1980) and that our samples were 10 cm distant to each other, we assume the independence of our data. Furthermore, no prominent changes in nematode community composition and densities were observed between the controls (AQ and IC), whereas indeed the lack of changes in community composition and densities in the IC treatments confirms the absence of enclosure effect in our experiment.

### 4.2. General colonisation pattern

The nematode assemblage colonising the defaunated sediments was very different from the assemblage in the controls and changing over time. The success of a single species, *Enoplolaimus littoralis*, suggests a species-specific response, rejecting our null first hypothesis ( $H_1$ ). Initially in both azoic and *Scolecopsis* treatments, a similar colonisation pattern was observed in terms of density, diversity and community composition, at least during the first week of the experiment. At first (i.e., at day 2), many species were able to colonise the newly available habitat as a result of an active infaunal lateral migration (Schratzberger et al., 2004; Gallucci et al., 2008). Passive dispersal caused by mimicking tides was precluded from the experimental design by guaranteeing that the water column never surpassed the edges of the experimental cores.

The initial rapid colonisation after 2 days was reflected in high species richness and the highest diversity ( $H'$ ) throughout the course of the experiment. The burning of the sediment to make it azoic may temporarily destroy the natural chemical and microbial community stratification of the sediment (Langezaal et al., 2004; Stocum and Plante, 2006), but it seems not to limit the entrance of nematodes into the defaunated cores. Hence, it suggests random migration of nematodes, supporting earlier findings (Schratzberger et al., 2004). At day 7, significant decreases in richness and diversity were



**Fig. 5.** Non-parametric multi-dimensional scaling ordination based on fourth-root transformed species density using Bray–Curtis similarity comparing nematode community among lab controls (open symbols) and treatments (AQ: triangles, IC: diamonds, AT: squares, ST: circles) over time (day-2: light grey, day-7: dark grey, day-14: medium grey; day-21: black).

**Table 2A**

Results from two-way PERMANOVA using Bray–Curtis similarity on fourth-root transformed data showing the effect of treatment × time on nematode community.

Two-way PERMANOVA	MS	Pseudo-F	<i>p</i>
Treatment <sub>(3,31)</sub>	4637	3.04	0.008
Time <sub>(3,31)</sub>	2616	4.45	<0.001
Treatment × time <sub>(9,31)</sub>	1531	2.61	<0.001

**Table 2B**

Pairwise tests showing differences between treatments at each time interval and between times at each treatment. Abbreviations as used in Table 1.

Pair-wise	2-day		7-day		14-day		21-day	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
AQ × IC	0.92	0.517	1.10	0.333	1.34	0.177	0.89	0.543
AQ × AT	2.33	0.020	3.04	0.010	2.57	0.020	2.46	0.015
AQ × ST	2.13	0.025	2.10	0.060	1.84	0.052	1.85	0.054
IC × AT	2.17	0.023	2.99	0.007	2.36	0.025	2.35	0.024
IC × ST	2.00	0.036	2.15	0.051	1.67	0.066	1.72	0.073
AT × ST	0.70	0.728	0.82	0.576	1.55	0.108	1.19	0.263
	AQ		IC		AT		ST	
	t	p(MC)	t	p(MC)	t	p(MC)	t	p(MC)
2 × 7	1.41	0.152	1.34	0.177	2.50	0.018	1.69	0.118
7 × 14	1.69	0.091	1.10	0.346	1.79	0.070	1.09	0.375
14 × 21	1.10	0.312	0.96	0.499	1.58	0.115	1.23	0.241

observed indicating a second step of the colonisation process. Possibly the chemical unattractiveness of the sediment had become a barrier for the survival or maintenance of the initially rapid-colonising species, so that now new colonisers were arriving and replacing them. At this time, the species, *Enoplolaimus litoralis*, showed high densities (92% and 87% of the total density in the azoic and *Scolecopsis* treatments, respectively). Although *E. litoralis* is an opportunistic species (Bongers et al., 1991) and a successful coloniser of oil-spill disturbed sediments (Giere, 1979), it is initially difficult to explain its success. Reproduction could be a possible explanation, but since the contribution of juveniles to the population structure was less than 40%, it is unlikely that the success of *E. litoralis* can be attributed solely to its reproductive activities. *Enoplolaimus litoralis* is assumed to be a predacious nematode, because of its large buccal cavity and associated teeth and mandibles, and therefore may be less dependent on the biochemistry of the sediment, compared to detritivorous or microvorous species. Hence, it seems to be less hampered in colonising the available sediments than are other species. Therefore, the colonisation success of *E. litoralis* likely results from the combination of its high mobility and feeding activity. This species may also be responsible for the reduction in diversity, by preying on other non-predatory species (i.e., a top-down effect). Top-down effects of predatory nematodes have been shown to control the density and species composition of nematode communities (Moens et al., 2000; Steyaert et al., 2001; Gallucci et al., 2005; Dos Santos and Moens, 2011).

From day 14 onwards, there was a slight increase in richness and diversity in both treatments. This could indicate better sediment conditions developed through colonisation of the sediment by microbes and diatoms from the water column. Although the recovery of the initial composition of bacterial community may occur 25 days after defaunation, the highest density of microbial cells may be found on around the tenth day of a microbial colonisation process (Stocum and Plante, 2006).

**Table 3**

Output of two-way crossed SIMPER analysis showing the top 50% typical species for each treatment. Overall similarity of each treatment is shown between brackets. Abbreviations as used in Table 1.

Species	AQ (72%)	IC (69%)	AT (63%)	ST (63%)
<i>Sigmaphoranema rufum</i>	10	9		
<i>Enoplolaimus litoralis</i>	8	8	32	20
<i>Mesacanthion</i> sp. 1	7	8	10	10
<i>Oncholaimellus calvadosicus</i>	7	6		7
<i>Ascolaimus elongatus</i>	6		10	9
<i>Chromadora axi</i>	6	6		
<i>Metadesmolaimus</i> sp. 1	6	6		
<i>Neochromadora munita</i>	6	5		
<i>Daptonema normandicus</i>		5		10

#### 4.3. Community shifts in the presence and absence of *Scolecopsis squamata* after 2 weeks of colonisation

Significant differences in nematode diversity and the density of *Enoplolaimus litoralis* between the azoic treatment and the *Scolecopsis* treatment occurred at day 14, and indeed PERMANOVA indicated the lowest non-significant difference in nematode community composition between these treatments at this time. These results indicate that the presence of *S. squamata* affects the colonisation, rejecting our second null hypothesis ( $H_2$ ).

In the presence of the polychaete, *Enoplolaimus litoralis* appeared in lower densities, from day 14 compared to when *Scolecopsis squamata* was absent. The polychaete seem to reduce the top-down effect of the predatory nematode, favouring earlier establishment of additional non-predatory species (i.e., *Daptonema normandicus*), which in turn led to an increase in diversity ( $H'$ ). In addition to delaying/inhibiting the colonisation of *E. litoralis*, the polychaete may also help in enhancing the environmental conditions, again contributing to the higher nematode diversity in the *Scolecopsis* treatment. Better environmental conditions can result from the polychaete's activities, which change the sediment chemistry through bioturbation and/or increase microbial metabolism by deposition of pseudofaeces (Dauer, 1983; Hartmann-Schröder, 1996; Pardo and Amaral, 2004; Van Hoey et al., 2004). On the other hand, polychaete bioturbation stimulates organic-matter mineralization via microbial degradation, by increasing the oxygen supply from its burrow construction and irrigation (Mermillod-Blondin et al., 2004; Papaspyrou et al., 2007; Timmermann et al., 2008; Braeckman et al., 2010). The individuals of *S. squamata* built temporary vertical tubes and occasionally burrow to a depth of 40 cm below the sediment surface (Hartmann-Schröder, 1996; Van Hoey et al., 2004). Indeed, during the sampling times, some burrows were found at the bottom of the cores, indicating activity of the polychaete.

#### 4.4. Succession patterns in contrasting environments

Three generalized models of community recovery were proposed by Connell and Slatyer (1977), based on interactions between pioneering species and later colonists: 1- Facilitation model: early colonists (i.e., pioneering) can promote the establishment of later colonists; 2- Inhibition model: early colonists reduce the establishment of later colonists; and 3- Tolerance model: early colonists have little or no effect on the establishment of later colonists. In mudflats, an environment with low hydrodynamic stress, very fine sediment and naturally high abundance of microphytobenthos (Reise, 1985), opportunistic epistrate-feeding nematode species dominate in the absence of macrofauna. This is explained by the high availability of microphytobenthos in the absence of grazing macrofauna (Van Colen et al., 2009). In this case, early-colonising nematodes contributed to a delay in the nematode community recovery, and for that reason an inhibition model was recognized. But in plots where macrofauna was still present, epistrate nematodes did not reach high densities, suggesting that their low densities provide stability for the nematode community (Van Colen et al., 2009).

In sandy beaches, an environment that sharply diverges from a mudflat because of the stronger hydrodynamics, coarse sediments and low abundance of microphytobenthos (McLachlan and Brown, 2006), the presence of macrobenthos seems to lead to a delay/inhibition of the establishment of high densities of opportunistic species, here represented by *Enoplolaimus litoralis*. The low density of dominant predatory nematode species favours the earlier establishment of secondary species (Vanaverbeke et al., 2007). In our experiment, in the absence of the polychaete, the



(continued)

	FC	Day-2				Day-7				Day-14				Day-21			
		AQ	IC	AT	ST	AQ	IC	AT	ST	AQ	IC	AT	ST	AQ	IC	AT	ST
<i>Spilophorella</i> sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Stephanolaimus</i> sp. 1	3	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Terschellingia</i> sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thalassironus</i> sp. 1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Theristus</i> sp. 2	15	6	5	1	2	6	4	0	2	10	2	0	0	10	5	0	17
<i>Trefusia</i> sp. 1	6	3	3	0	0	0	1	0	0	0	1	0	0	2	2	0	5
<i>Trichotheristus mirabilis</i>	25	21	9	0	1	16	8	4	2	8	3	0	6	4	4	8	2
<i>Trichotheristus</i> sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Tripyloides</i> sp. 1	0	0	0	0	0	0	0	0	0	1	2	0	0	3	0	0	2
<i>Trissonchulus</i> sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Xyala striata</i>	45	12	13	0	0	2	13	0	2	7	4	0	1	2	10	43	9

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