Chapter VI

The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments

Results presented as

Steyaert M, Moodley L, Vanaverbeke J, Vandewiele S, Vincx M (submitted) The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments

Abstract

The direct role of oxygen in structuring the vertical distribution of an intertidal nematode community was investigated in manipulation experiments with sediments collected from the Oosterschelde (The Netherlands). In these experiments, the vertical distribution of the nematode species was examined in response to sediment inversion with or without oxygen.
The introduction of oxygen to deep layers significantly altered the vertical distribution of the nematode densities; highest numbers were recorded in the artificially aerated bottom layer. However, the distribution of the majority of the species (~ 79 %) was not directly linked to oxygenation. Dominant species were *Terschellingia communis, Microlaimus tenuispiculum* and *Sabatieria pulchra*. Only a small fraction (~ 2 %) of the nematode community was restricted to oxygenated sediment layers, irrespective of sediment depth. Six species belonged to this group (*Aegialoalaimus elegans, Axonolaimus helgolandicus, Bathyeurystomina sp., Desmoscolex sp., Dorylaimopsis sp.* and *Monoposthia mirabilis,*) and their distribution was evidently directly governed by oxygenation. The majority of the species examined are highly tolerant to short-term anoxic conditions.

Introduction

Vertical distribution patterns of nematodes are often species-specific and established as a response to biogeochemical properties of the sediment (Jensen 1981, 1987, Bouwman *et al.* 1984, Platt & Lambshead 1985, Jensen & Aagaard 1992, Hendelberg & Jensen 1993, Steyaert *et al.* 1999). The oxygen supply of the pore water is many times suggested as the predominant structuring factor, coupled to a complex system of interacting environmental factors. However, the mechanistic understanding of the relationship between oxygen with nematode distribution is limited.

Surface dwellers as well as deep infaunal occurring species prevail in all sediment types, ranging from coarse sands to fine mud. Fine sediments harbour in general more surface dominating species (*e.g. Daptonema tenuispiculum, Ptycholaimellus ponticus*), which are primarily directed by steep oxic-sulphidic gradients. Subsurface distribution may be coupled to an indirect positive effect of low oxygen (Neira *et al.* 2001) through decreasing predatory and competitive activity, and preservation of organic matter leading to high food availability and quality. Bioturbating processes have a massive influence on population structure as oxygen and particulate organic matter are episodically inserted deeper down in the sediment. Given their aerobic respiration, the effect of bioturbation may especially be of relevance for deep infaunal nematode species of fine-grained sediments. In coarse, well-aerated sediments, biotic interactions may be rather important in structuring nematode communities *e.g.* species are vertically segregated in order to minimise competitive or predative interactions (Joint *et al.* 1982, Steyaert *et al.* 2003).

The oxygen availability of sediments influences structural as well as functional aspects of the nematode communities (Vanaverbeke *et al.* 2003). Due to their small size and short generation times, their benthic life cycle and their ubiquitous distribution, nematodes are potential biological monitors to evaluate oxygen depletion in intertidal areas.

The direct role of oxygen on the vertical distribution of nematodes was investigated by means of experiments in which different oxygen conditions were imposed on sediments from the Oosterschelde (The Netherlands). The migratory activity of the nematode community and of nematode species was evaluated as function of oxygen availability. The null-hypothesis to be tested is that the vertical distribution of the nematode assemblages and nematode species is not influenced by changing oxygen conditions (*e.g.* nematodes do not migrate to favourable oxygen conditions).

Material and methods

Sediments (mean median grain size 92 µm) were collected on 8th March 2002 from an intertidal area (51°32.0'N; 03°52.5'E) of the Oosterschelde (The Netherlands) at low tide. Perspex cores (i.d. 3.6 cm) were used to sample the sediment up to a depth of 5 cm. Cores were transported to the lab and kept in the dark at 12°C. The cores were filled with fresh Oosterschelde water and continuously aerated. This was the control treatment (two C-cores). In two cores (An-cores), the sediment was turned upside down, imposing anoxic conditions on the original 0-1 cm depth layers. Similarly, in two other cores (Ox-cores), the sediment was inverted but with the presence of an air flushed silicone tubing running through the bottom of the core. Inversion of the sediment enabled to distinguish between oxygen-mediated and food-mediated interactions. Oxygen diffuses through silicone, thereby introducing oxygen to this otherwise anoxic sediment (Moodley *et al.* 1998). In all manipulated cores (Ox-cores and An-cores), the original 4-5 cm depth layer became the surface layer, oxygenated by the aerated overlying water (Fig. 1). Extra food was added (approximately 2 mg diatom C) on top of the C-cores and at the bottom of each manipulated core. All cores were incubated in the dark for one week. Separate cores were used to measure sediment oxygen concentration and redox potential after one week incubation, using micro-electrodes (UNISENSE). At the end of the experiment, cores were sliced in 1 cm slices and stored in a neutral 4% hot formaldehyde solution. The top layer of 2 mm was sampled with a pipette. The presence of living nematodes was verified prior to preservation.

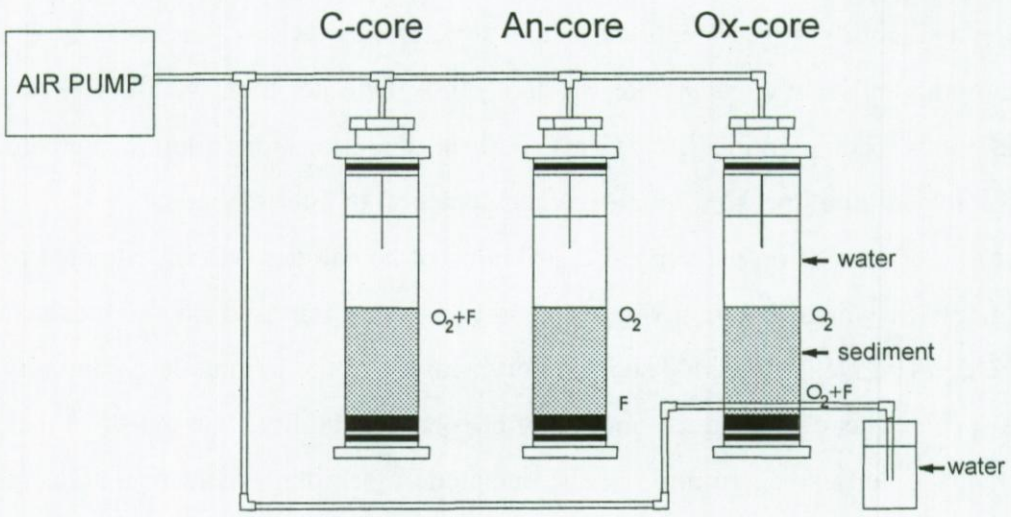

Figure 1. Schematic of the experimental set-up. The water in the cores was continuously bubbled with air and circulated. O_2 and F indicate were oxygen and food is added. One replicate of each treatment is shown.

Nematodes were extracted from the sediment by centrifugation with Ludox (Heip *et al.* 1985) and stained with Rose Bengal. Nematodes were enumerated from all slices. Identification to species level was done for the upper (0-1 cm) and the deepest (4-5 cm) sediment layer on the first 120 nematodes, mounted into Cobb-slides. For comparison with field nematode density profiles, two cores were sliced and processed, as described, directly upon sampling.

To test for variation in total nematode densities with treatment, with depth and with treatment × depth, a univariate 2-way analysis of variance (ANOVA) was used. A 'split-plot' design was constructed with replicates nested within treatments, following Steyaert *et al.* (2001). All data were log (x+1) transformed prior to analysis in order to met ANOVA assumptions.

Ordination techniques from the PC-ORD for Windows package (version 4.20, McCune & Mefford 1999) were used to examine similarities between 0-1 and 4-5 cm layers of the different treatments, based on the species composition of the communities. A Detrended Correspondence Analysis (DCA) was applied for the determination of the length of gradient, followed by a Correspondence Analysis (CA) using non-transformed relative abundances. Species rarer than $F_{max}/5$ (F_{max} is the frequency of the commonest species) were down-weighted in proportion to their frequency. For all analyses, nematode values of the 0-1 cm surface layer included the upper 2 mm that was sampled separately.

Results

The oxygen profiles of the un-manipulated and the inverted sediment were similar; the oxygen content dropped from 305 µmol l^{-1} at the surface to 19.4 µmol l^{-1} at 250 µm sediment depth (Fig. 2).

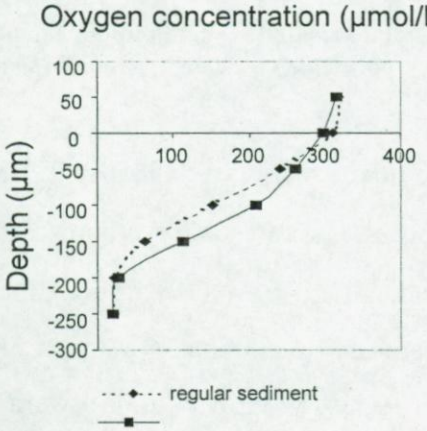

Figure 2. Oxygen profiles in un-manipulated and inverted sediment.

Sediment redox values of both the un-manipulated and the inverted sediment indicated that only the uppermost 2 mm was oxidised, evidenced as a light coloured top layer (Fig. 3). The surface layers of An-cores and Ox-cores (the original 4-5 cm layer) were initially dark brown and smelled strongly of sulphide. Oxygen profiles were not made around the silicone tube (Ox-core), but a clear oxidised layer of approximately 2 mm was clearly visible around the tubing during slicing.

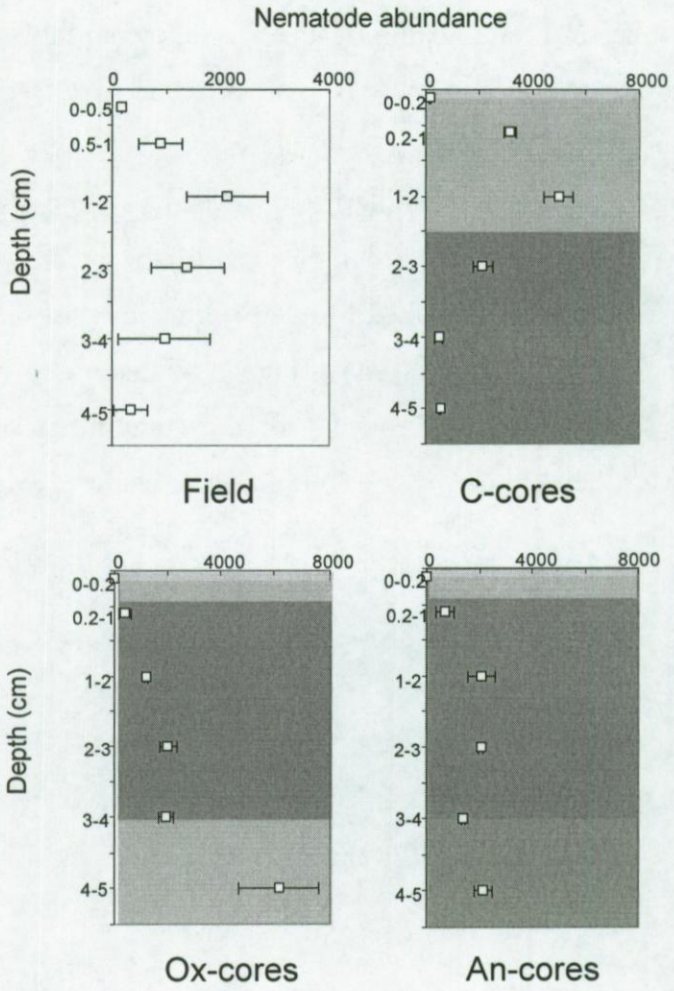

Figure 3. Depth distribution of the total nematode community for the field situation and the different treatments (shading represents visual observation of sediment colour).

Total nematode abundance of the field situation was 5850 ± 971 ind. 10 cm^{-2}. After one week, total nematode abundance of C-cores, Ox-cores and An-cores were respectively 11550 ± 305, 11820 ± 906 and 8390 ± 440 ind. 10 cm^{-2}. ANOVA 'split-plot' analysis demonstrated that nematode densities numbers between treatments ($F_{2,2}$ = 4.68; $p > 0.05$) and between treatments and field situation ($F_{3,3}$ = 1.69; $p > 0.05$) were not significantly different.

Investigation of the different life stages (juveniles/ females/ males) revealed no changes of juveniles with time.

Similar to field situation, in the C-cores, a subsurface density peak was recorded at a depth of 1-2 cm (Fig. 3). Densities decreased gradually deeper down the sediment. ANOVA 'split-plot' analysis demonstrated no significant differences between nematode depth distribution of the C-cores and the field situation ($F_{4,12} = 0.449$; $p > 0.05$). The distributional pattern in the An-cores showed no obvious peak. In the Ox-cores, peak values were recorded in the 4-5 cm sediment layer (the original surface layer). Total nematode distribution changed significantly in depth ($F_{5,5} = 73.89$; $p < 0.001$) for all cases. The interaction between treatment and depth was significant ($F_{10,15} = 18.56$; $p < 0.001$). The distributional patterns of total nematode community were significantly different between the three sets of cores.

In total 44 species were distinguished: 31, 33 and 32 in respectively the C-cores, Ox-cores and An-cores (Table 1). The species from the different cores were categorized according to their presence/absence in the upper (0-1 cm) and/or deeper (4-5 cm) sediment layer.

	C-cores			Ox-cores			An-cores		
Number of species	0-1		4-5	0-1		4-5	0-1		4-5
Total	27	(31)	16	29	(33)	20	26	(30)	23
Common in both layers		12			13			19	
Only present in 0-1		15*			16*			9*	
Only present in 4-5		4			4*			5	
Relative abundance									
Total		100.0			100.0			100.0	
Common in both layers		79.3			94.7			95.0	
Only present in 0-1		19.3*			0.7*			1.6*	
Only present in 4-5		1.4			4.6*			3.4	

Table 1. Occurrence of species in top and/or bottom layers of the different treatments (*: species present in oxygenated layers).

Irrespective of the treatments in the cores, two striking features were evident (Table 1): (1) only a minor fraction of the nematode communities (4 – 5 species or 1.4 - 4.6 %), were confined to the 4-5 cm layer, irrespective of oxygenation; (2) the largest fraction, 79 - 95 % of the nematode community occurred both in the upper and deeper layers, irrespective of oxygenation. In order to identify species that were rigorously restricted to oxygenated areas, the species confined to oxygenated layers within a treatment (4 species in the 4-5 cm layer of the Ox-cores and 15 species in the 0-1 cm layer of the C-cores, Table 2), were examined for their occurrence in anoxic layers. In total, 6 species, *Aegialoalaimus elegans* (De Man 1907), *Axonolaimus helgolandicus* (Lorenzen 1971), *Bathyeurystomina sp.*, *Desmoscolex sp.*,

Dorylaimopsis sp. and *Monoposthia mirabilis* (Schulz 1932), were restricted to these oxic horizons.

	C-core		Ox-core		An-core	
	0-1	4-5	0-1	4-5	0-1	4-5
Ox-core: 4-5 cm						
Anoplostoma viviparum	*			*	*	*
Axonolaimus helgolandicus				*		
Dorylaimopsis sp.				*		
Monoposthia mirabilis	*			*		
C-core: 0-1 cm						
Aegialoalaimus elegans	*					
Anoplostoma viviparum	*			*	*	*
Bathyeurystomina sp.	*					
Chromadora macrolaima	*		*	*	*	*
Chromadorella circumflexa	*				*	*
Chromadorella sp.	*		*			*
Chromadorita sp.	*		*	*	*	*
Cyartonema sp.	*					*
Desmoscolex sp.	*					
Eleurolaimus sp.	*		*		*	*
Halalaimus sp.	*		*		*	
Hypodontophora sp.	*				*	*
Monoposthia mirabilis	*			*		
Sphaerolaimus sp.	*		*			
Viscosia franzii	*		*	*	*	*

Table 2. Presence of species from (a) the 4-5 cm layer of the Ox-cores and (b) the 0-1 cm layer of the C-cores, in other treatments.

	C-core		Ox-core		An-core	
	0-1	4-5	0-1	4-5	0-1	4-5
C-core: 4-5 cm						
Daptonema sp.		*	*			
Metalinhomoeus sp.		*			*	
Siphonolaimus sp.		*				
Spirinia parasitifera		*	*	*	*	
An-core: 4-5 cm						
Calyptronema sp.						*
Chromadorella sp.	*		*			*
Chromadorella circumflexa	*				*	*
Cyartonema sp.	*					*
Theristus sp.						*

Table 3. Presence of species from (a) the 4-5 cm layer of the C-cores and (b) the 4-5 cm layer of the An-cores, in other treatments.

A similar analysis for the possible preference for anoxic layers revealed that only 3 species, namely *Calyptronema sp.*, *Siphonolaimus sp.* and *Theristus sp.*, are strictly confounded to anoxic horizons (Table 3). The remaining 35 species are recorded in both oxic and anoxic horizons of the different treatments. Most abundant species were *Terschellingia communis* (De Man 1888), *Microlaimus tenuispiculum* (De Man 1922) and *Sabatieria pulchra* (Schneider 1906) totally accounting for about 45 % of the nematode assemblage.

Similarities between of 0-1 and 4-5 cm sediment layers from different treatments are shown in the CA plot (Fig. 4). The first ordination axis had an eigenvalue of 0.309; the second and the third (not depicted) axes had very low eigenvalues of respectively 0.141 and 0.126.

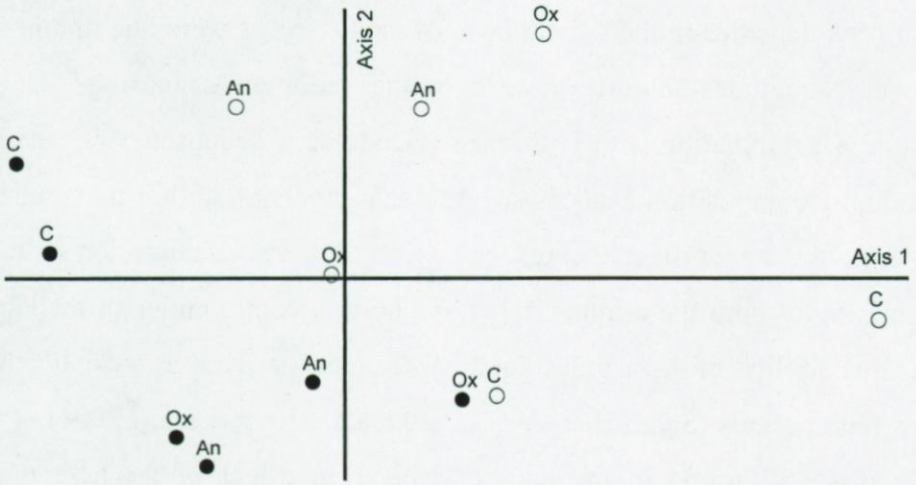

Figure 4. Results of Correspondence Analysis. C: C-cores, Ox: Ox-cores, An: An-cores. Full circles: 0-1 cm layers, open circles: 4-5 cm layers.

Along the first axis, samples of the 0-1 cm layer of the C-cores are clearly separated from the 4-5 cm layer of the C-cores (only one replicate). All samples of manipulated cores and one replicate sample of the 4-5 layer of the C-cores are plotted in between. The analysis further indicated the similarity between all original upper layers and all original deeper layers, since 0-1 layers of the C-cores and 4-5 layers of the An-cores and Ox-cores are plotted on one side of axis 2; 4-5 layers of the C-cores and 0-1 layers of the An-cores and Ox-cores on the other side of axis 2. Characteristic species for the upper sediment layer of the C-cores were *Bathyeurystomina* sp., *A. elegans*, *Desmoscolex* sp. and *Sphaerolaimus* sp.; for the 4-5 cm layer of the C-cores *Siphonolaimus* sp., *Daptonema* sp., *Metalinhomoeus* sp..

Discussion

Oxygen profiles were not measured around the silicone tube but the presence of the oxidised layer around it confirmed the introduction of oxygen into this layer. Sediments in the experiment were not sieved or homogenised so that an unequal number of species may be attributed to patchiness. Additionally, macrofauna possibly present may have influenced the distribution patterns by introducing oxygen to deeper sediment layers (*e.g.* Wetzel *et al.* 1995, Fenchel 1996). Irrespective, it was evident that oxygenated sediments represent favourable conditions for nematodes; nematodes were concentrated in the oxygenated bottom layer. Both in the field situation and in the control cores, there was a subsurface peak in densities suggesting that peak densities in the 4-5 cm layer of the Ox-cores were due to migration from the layer above. Nematodes migrate upward in the sediment as a response to oxygen deficiency, often in combination with H_2S stress (Hendelberg & Jensen 1993 and references therein). Community composition analysis suggests that inversion and/or manipulation of the oxygen regime in the experimental cores gave rise to vertical migration of nematodes, resulting in a mixing of both the original upper and bottom communities of the experimental cores. Despite the ability of nematodes to migrate actively over a wide depth range in relatively short time periods (Schratzberger *et al.* 2000a,b, Steyaert *et al.* 2001), a fraction of the nematodes was still found in the anoxic layers (but effect of possible macrofauna). Evidence for a slow recovery (years) of nematode assemblages after a hypoxic period was given by Austen *et al.* (1991).

In the experiment most nematode species (79 %) had a ubiquitous distribution relative to oxygen availability (group 2) and are evidently tolerant to short-term anoxic conditions and simultaneously also burial. This partly explains the minor changes in nematode community composition when sediment cores were inverted. The dominance of this group of species can be linked to the nature of the sediment. In general, fine sediments with steep gradients of oxygen and toxic sulphide harbour less sensitive species compared to well-aerated coarse sandy sediments (Steyaert *et al.* 1999, Vanaverbeke *et al.* 2003). The dominant species prevailing at our sampling station (*Terschellingia communis*, *Microlaimus tenuispiculum* and *Sabatieria pulchra)* are characteristic species for muddy suboxic or anoxic environments (Warwick & Gee 1984, Ólafsson 1992, Hendelberg & Jensen 1993, Modig & Ólafsson 1998, Boyd *et al.* 2000, Schratzberger *et al.* 2000a, Wetzel *et al.* 2002). In contrast, *Monoposthia* and *Desmoscolex*, which were only recorded in oxygenated conditions in the experiment, are

common in well-aerated sandy sediments (*e.g.* Kim & Shirayama 2001, Gheskiere *et al.* 2002). In this experiment, the occurrence of *Monoposthia mirabilis, Desmoscolex sp.* and 4 other species, *Aegialoalaimus elegans*, *Axonolaimus helgolandicus, Bathyeurystomina sp.* and *Dorylaimopsis sp.*, seemed to be directly determined by the oxygen supply and may be potential bio-indicators of oxygenated intertidal sediments. The third group of species, defined as species which were not recorded in oxic environments, included *Calyptronema sp., Siphonolaimus sp.* and *Theristus sp.*. There are however, no reports of these species being strict anaerobes and we have no explanation for their confinement to anoxic sediment layers.

Oxygenated sediment layers are clearly favourable habitats for nematodes and therefore, the subsurface peaks of nematodes generally found in intertidal sediments (Soetaert *et al.* 1994) reflect other structuring factors. This may include inter-intra specific competition, resource partitioning, reducing risks of predation *etc.*

Similar experiments with coarse sandy, well-aerated sediments and associated nematode communities may provide more insight into the direct role of oxygen. It is expected that species like, *Richtersia* sp. or *Desmoscolex* sp., typical of sandy sediments may be more sensitive to anoxic conditions. Experiments incubating known densities of potential oxyphilic species in a set of different oxygen regimes could elaborate on the controlling role of oxygen.

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