Biodivers Conserv (2009) 18:33–54
DOI 10.1007/s10531-008-9433-1

ORIGINAL PAPER

Biodiversity response to experimental induced hypoxic-anoxic conditions in seagrass sediments

Cristina Gambi · Silvia Bianchelli · Marta Pérez · Olga Invers · Joan Manuel Ruiz · Roberto Danovaro

Received: 5 November 2007 / Accepted: 26 June 2008 / Published online: 9 July 2008

Abstract The effects of induced hypoxic-anoxic conditions on the metazoan meiofaunal assemblages and nematode diversity were investigated with an in situ experiment in a *Posidonia oceanica* meadow. The experiment, of the duration of five months, was performed in three experimental sets of plots. Two of them were enriched with organic matter to induce anoxic conditions (1 set with sucrose and 1 set with sugar plus nutrients, i.e. nitrogen and phosphorus) whereas the last set of plots was kept undisturbed and used as Control. Metazoan meiofauna displayed a fast response to the induced anoxic conditions with an immediate reduction of the richness of taxa (only nematodes and copepods tolerated the hypoxic-anoxic conditions). Nematodes were the most tolerant organisms as their species richness did not change in hypoxic-anoxic conditions, but their species composition and trophic structure displayed significant changes. Some genera (*Desmoscolex* and *Bolbolaimus*) were replaced by other (*Chromadorella*, *Sabatiera* and *Polysigma*) more tolerant to the extreme conditions. No significant differences were observed in the Control plots, whereas in treated plots, selective deposit feeders and predators decreased significantly, being replaced by non-selective deposit feeders and epistrate feeders. These results indicate that, events causing a reduction in oxygen availability, can have an impact on the nematode beta-diversity and functional diversity with potential important implications on the benthic food web and functioning of the seagrass systems.

Keywords Biodiversity · Hypoxic-anoxic conditions · Metazoan meiofauna · Nematodes · *Posidonia oceanica*

C. Gambi · S. Bianchelli · R. Danovaro (✉)
Department of Marine Sciences, Polytechnic University of Marche, Via Brecce Bianche, 60131 Ancona, Italy
e-mail: r.danovaro@univpm.it

M. Pérez · O. Invers
Departament d'Ecologia, Universitat de Barcelona, Av.da Diagonal 645, 08028 Barcelona, Spain

J. M. Ruiz
Instituto Español de Oceanografía, Magallanes 2, Apdo. 22, 30740 San Pedro del Pinatar, Spain

Introduction

Coastal eutrophication is being recognized as one of the most important emerging problems and, during the past four decades, has exponentially increased in intensity, geographic extension and environmental consequences (Nixon 1995; Jørgensen and Richardson 1996; Cloern 2001; Livingston 2001; Painting et al. 2007). Eutrophication is typically related with the increase of nutrient and organic matter loads, which could induce a progressive reduction of oxygen availability (Cloern 2001) due to large amounts of organic matter derived from primary production (Danovaro 2003). Higher rates of microbial decomposition can deplete dissolved oxygen near the sediment-water interface and produce H_2S that enters the water column (Diaz and Rosenberg 1995). Under strong water column stratification or stagnation, hypoxia or anoxia can persist for long enough to cause the mortality of benthic animals (Pearson and Rosenberg 1978; Brown et al. 1987; Tutsumi et al. 1991; Pocklington et al. 1994; Wu et al. 1994; Karakassis et al. 2000; Grall and Chauvaud 2002; Gray et al. 2002). Therefore, sediments and benthic communities appear the most sensitive compartment of coastal ecosystem to eutrophication and hypoxia (Jørgensen and Richardson 1996; Powers et al. 2005). Hypoxia influences benthic organisms directly altering their metabolic processes and mobility but also indirectly modifying community structure, biodiversity and relationships among species and trophic groups (Diaz and Rosenberg 1995; Modig and Olafsson 1998; Peterson et al. 2000; Powers et al. 2005). Even if many macrobenthic organisms survive short term hypoxia through behavioral or physiological adaptations, mass mortality of some benthic species generally occurs, depending on the magnitude of oxygen depletion (Grall and Chauvaud 2002). Larger long-lived species are eliminated first, then communities shift towards dominance by small, short-lived, often opportunistic species in successive stages depending on the frequency and intensity of hypoxia (Diaz and Rosenberg 1995).

The direct effects of hypoxic-anoxic events on benthic macrofauna are well-document (Kristensen 2000; Nordberg et al. 2001; Hansen et al. 2002) whereas little is known for meiofauna (Josefson and Widbom 1988; Murell and Fleeger 1989; Hendelberg and Jensen 1993; Steyaert et al. 2007). Meiofauna due to their relatively short life cycles, high turnover rates and lack of larval dispersion, are expected to respond rapidly to environmental changes and food availability (Modig and Olafsson 1998; Danovaro and Fabiano 1997; Danovaro et al. 1995a, 2000a, b, 2004; La Rosa et al. 2001; Danovaro and Gambi 2002; Austen and Widdicombe 2006; De Troch et al. 2006) whereas macrofauna respond more slowly (Somerfield et al. 1995; Albertelli et al. 1999; Widdicombe and Austen 2001; Austen and Widdicombe 2006). Nematodes, the dominant meiofaunal taxon (>50% of metazoan meiofauna in the coastal areas, Coull 1988), have been largely utilized as indicators of organic disturbance because their ubiquity, high abundance and high taxonomic diversity (Bongers and Ferris 1999; Mazzola et al. 1999; Mirto et al. 2002; Vanaverbeke et al. 2004; Fraschetti et al. 2006) and are known to persist and increase their relative importance under long periods of hypoxic-anoxic conditions (Heip et al. 1985; Meyers et al. 1987; Vopel et al. 1996; Modig and Olafsson 1998).

The aims of the present study are to investigate the effects of hypoxic-anoxic conditions (*sensu* Pearson and Rosemberg 1978) on metazoan meiofaunal assemblages and nematode diversity inhabiting *Posidonia oceanica* sediments by means of in situ experiments. We hypothesize that these conditions can immediately modify the meiofaunal assemblages and nematode community composition inhabiting a *P. oceanica* meadow. *P. oceanica* is known as feeding and nursery grounds for many fish species (Nagelkerken et al. 2000) therefore the effects of hypoxic conditions on benthic components within the seagrass can have severe

consequences on the higher trophic levels of these coastal ecosystems (Green and Short 2003). Benthic invertebrates, both macrofauna and meiofauna, represent an important and easily accessible prey resource for demersal fishes and crabs (thus becoming a main pathway of energy transfer to higher trophic levels; Danovaro et al. 1995b; Leguerrier et al. 2003), therefore factors influencing benthic populations can alter the production at higher trophic levels (i.e. demersal fishes; Peterson et al. 2000; Powers et al. 2005) with important potential implications on biodiversity and conservation of these benthic ecosystems.

Methods

Experimental design and sampling

The effects of experimentally induced anoxia on meiobenthic communities have been previously investigated using laboratory incubation (Modig and Olafsson 1998; Widdicombe and Austen 2001; Steyaert et al. 2007), here we report the results of in situ manipulations. The experiments were carried out in a dense *P. oceanica* meadow of the Medes Islands (NE Spain, Fig. 1). The sampling area was located 1 mile offshore at 10 m depth. Nine randomly selected plots (50 × 50 cm separated by 5 m) were permanently marked in the meadow (Fig. 2). Three of them were enriched with organic matter (i.e., Organic Matter treatment, OM) and periodically added with ca. 800 g of sucrose per plot in the form of solid caramel pieces. The sugar was added every week from the onset of the experiment for the first three months and every two weeks for the last two months. Three plots were enriched with sugar as above and nutrients (nitrogen and phosphorus; i.e., Organic Matter plus Nutrients treatment, OMN) using slow-release commercial fertilizers (final composition 125 g N and 125 g P per plot) every two weeks. The three remaining plots were kept undisturbed and considered as Control. The addition of organic matter (sucrose) and nutrients was performed according to previous field experiments to test the effects of the induced hypoxic conditions on seagrass meadows (Penhale and Wetzel 1983; Terrados et al. 1999; Holmer et al. 2005). During this field experiment, the OM and OMN

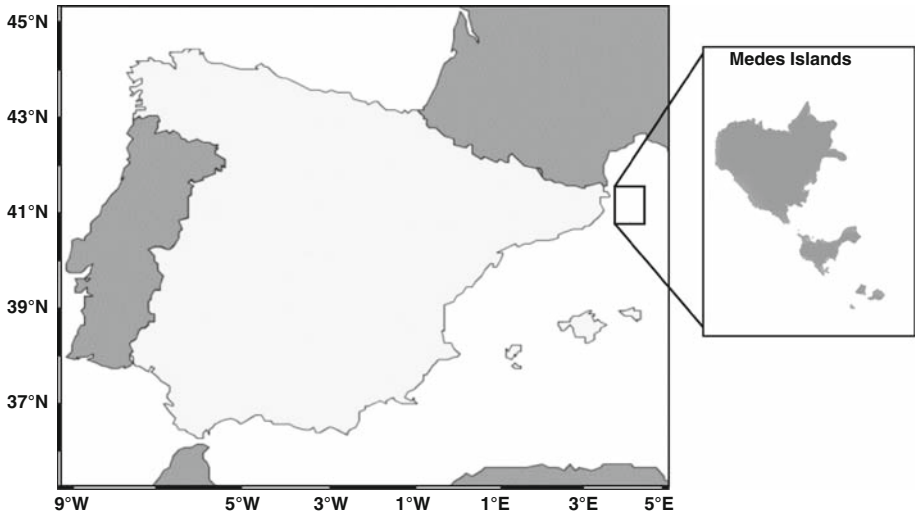

Fig. 1 Sampling area: location of the Medes Islands

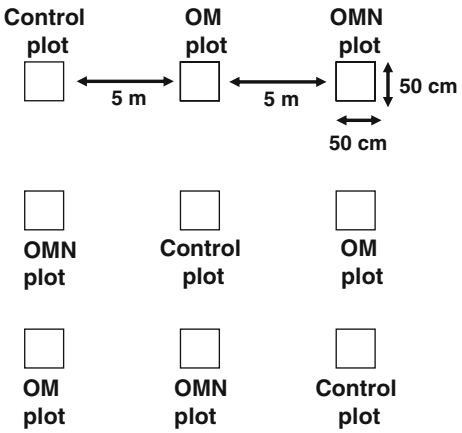

Fig. 2 Experiment design displaying the position of the Control, OM and OMN plots (not in scale)

plots were selected to investigate separately the effects of sucrose and sucrose plus nutrients on the metabolism of the *P. oceanica* and these results are reported in details in Pérez et al. (2007). For the aims of the present study, we considered the OM and OMN plots as plots where the hypoxic conditions were induced without hypothesizing a different response of meiofaunal assemblages to sucrose and sucrose plus nutrients treatments.

The experiment began in March 2002 and was carried out for five months. Pérez et al. (2007) reported that three months (intermediate sampling, May 2002) was sufficient to induce the hypoxic conditions in OM and OMN plots. In May and July, visual signs of reducing conditions in the sediment were observed in the treated plots compared to the Control. This allowed to investigate the effects of induced hypoxic-anoxic conditions on metazoan meiofaunal assemblages inhabiting the *P. oceanica* meadows (approximately for 3–4 months). Metazoan meiofauna were sampled only at the start and the end of experiment (March and July 2002, respectively).

Sediment corers (internal diameter 4 cm; containing live *P. oceanica* leaves and roots) were randomly collected in each experimental plot by scuba divers. Three replicate corers from each plot were preserved in zinc acetate for sulfur pools analyses and three cores from each set of plots were immediately kept frozen at −20°C for metazoan meiofaunal analyses.

Sediment sulfur pools analysis

For sulfur pools, sediments were distilled following a 2-step distillation procedure (Fossing and Jørgensen 1989) with the modification that the distillate was precipitated as Ag_2S instead of ZnS. The first step in the procedure uses HCl to derive the acid-volatile fraction which contains the pools of free H_2S, HS^- and FeS. In the next step, Cr^{2+} is added to obtain the chromium reducible sulfur pools consisting of FeS_2 and S^0. The size of the sulfur pools was determined by the weight of the precipitates (Frederiksen et al. 2008). Ammonium concentration in pore water samples was measured by the colorimetric method described by Koroleff (1983). Further details are reported in Pérez et al. (2007).

Meiofaunal and nematode analyses

For metazoan meiofaunal extraction, each sediment core was sectioned into different layers: 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10 cm. Sediment was sieved through

a 1000 μm and a 30 μm mesh, respectively, to retain the smallest organisms. The fraction remaining on the latter sieve was resuspended and centrifuged three times with Ludox HS 40 (density arranged to 1.18 g cm^{-3}) as described by Heip et al. (1985). All metazoan animals were counted and classified per taxon under a stereomicroscope using Delfuss cuvettes, after staining with Rose Bengal (0.5 g l^{-1}).

For diversity analysis, from the top 1 cm of each sediment core, ca 100 nematodes (or all nematodes if lower abundances were observed) were randomly withdrawn and mounted on slides following the formalin-ethanol-glycerol technique described by Seinhorst (1959) to prevent dehydration. All nematodes were identified to species level (whenever possible, due to the presence of several unknown species) according to Platt and Warwick (1983, 1988), Warwick et al. (1998) and the recent literature (Deprez and al 2005) dealing with new nematode genera and species.

The trophic structure was defined according to Wieser (1953). Nematodes were divided into four groupings as follows: (1A) no buccal cavity or a fine tubular one-selective (bacterial) feeders; (1B) large but unarmed buccal cavity-non selective deposit feeders; (2A) buccal-cavity with scraping tooth or teeth-epistrate or epigrowth (diatom) feeders; (2B) buccal cavity with large jaws-predators/omnivores.

Diversity indexes

Nematode diversity was estimated using Species Richness (SR) as the total number of species identified at each station. Since species richness is strongly affected by the sample size, in order to standardize the values of nematode diversity, the expected number of species E(X) was considered. At each site, the species-abundance data were converted into rarefaction diversity indices (Sanders 1968, as modified by Hulbert 1971). The expected number of species for a theoretical sample of 51 specimens, ES(51), was selected to compare our results with literature.

Species diversity was measured by Shannon-Wiener information function (H', using log-base 2), Margalef index: $(D = (S - 1)/\ln N)$, where S is the number of species and N is the number of individuals in the sample (Margalef 1958) and evenness as J' (Pielou 1975).

All the diversity indexes were performed using the software package PRIMER v5 program (Plymouth Marine Laboratory; Clarke 1993).

The Index of Trophic Diversity (ITD) was calculated as $ITD = {g_1}^2 + g_2^2 + g_3^2 \ldots + g_n^2$, where g is the relative contribution of each trophic group to the total number of individuals and n is the number of trophic groups (Gambi et al. 2003). For $n = 4$ (as in the present study) ITD ranges from 0.25 (high trophic diversity) to 1.00 (low trophic diversity).

Statistical analyses

A two-way analysis of variance (ANOVA, GMAV 5.0 software, University of Sidney, Australia) was used to test for differences in total metazoan meiofaunal abundance, total number of taxa, nematode species richness and functional diversity. Time (start vs. end of the experiment) and treatments (Control, OM and OMN plots) were considered as fixed factors with two and three levels, respectively. When significant differences were encountered, Student Newman-Keuls (SNK) *post-hoc* comparison tests (at $\alpha = 0.05$) were also carried out to identify among which sets of plots and when the significant differences occurred. Since the presence of the *P. oceanica* determined a high variability in meiofaunal abundance and diversity among the experimental plots at the start of the

experiment and did not allow to clearly detect the effects of the hypoxic-anoxic conditions, we estimated the shift of the investigated variables between the start and the end of the experiment in each experimental plot, normalising the change of each variable with the value observed at the start of the experiment.

A one-way analysis of variance (ANOVA) was used to test for differences for all of the variables listed above during the experiment in each plot. Prior to the analysis, the homogeneity of variance was tested by Cochrans' test and, when necessary, the data were appropriately transformed.

Cluster analysis was also carried out to ordinate plots enriched with organic matter and Control basing on nematode diversity. A ranked matrix of Bray-Curtis similarities was used as input for this test. To test the hypothesis that the similarities among the three types of experimental plots (Control, OM and OMN, respectively) changed between the start and the end of the experiment, mean dissimilarities among replicates were estimated. The turnover diversity (i.e., β-diversity estimated as % Bray-Curtis dissimilarity; Gray 2000) was estimated as the dissimilarity of species composition i) among the plots enriched with organic matter and Control at the start and the end of the experiment and ii) between the start and the end of the experiment in each plot trough the SIMPER analyses (based on the Bray-Curtis similarity index). All absolute data were presence/absence transformed prior to the analysis.

Pair-wise analysis of similarity, ANOSIM analysis, was performed to test for significant differences in nematode community composition among the different plots. Cluster, SIMPER and ANOSIM analyses were performed using the software package PRIMER v5 program (Plymouth Marine Laboratory; Clarke 1993).

Results

Sediment sulfur pool

In Control sediments, the pools of free H_2S, HS^- and FeS was very low whereas increased significantly ($P < 0.001$) after the three months from the start of the experiment in OM and OMN plots and differences further increased by the end of the experiment. Also, the chromium reducible sulfur pools remained low in Control sediments and increased significantly ($P < 0.05$) in OM and OMN plots at the end of the experiment. More details are reported in Pérez et al. (2007).

Meiofaunal assemblages

Total metazoan meiofaunal abundance displayed significant difference between the start and the end of the experiment in each plot and among the different plots at the end of the experiment (Fig. 3 and Table 1). The change (shift) of meiofaunal abundance observed between the start and the end of the experiment was consistent in each experimental plot, but was not statistically significant (Table 2).

Metazoan meiofaunal abundance decreased significantly from the surface to the deepest sediment layers in all plots, both at the start and at the end of the experiment (results of ANOVA with three factors not shown; Fig. 4a,b,c). Nematodes were always the most abundant taxon (77–100%) except in the top 0–1 cm layer of the Control and OMN plots at the start of the experiment, where copepods dominated (69% and 60% in Control and

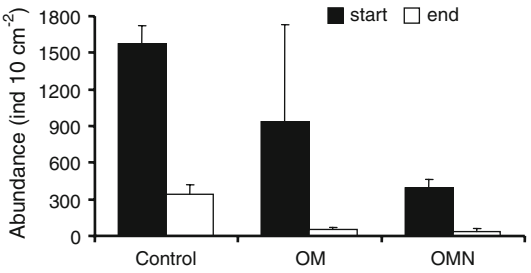

Fig. 3 Total meiofaunal abundance (ind. 10 cm^{-2}) in Control, OM and OMN plots at the start and the end of the experiment. Average values were calculated using three replicates and bars indicate the standard deviation

OMN plots, respectively). Other higher taxa (Polychaetes, Ostracods, Tardigrades, Amphipods, Tanaids, Bivalves and *incertae sedis*) contributed to the total community structure for $< 2\%$. The total number of higher taxa (including Nematodes and Copepods) was significantly higher at the start of the experiment in the OM and Control ranging from 5 to 9, and decreased to 2–3 higher taxa (Nematodes, Copepods and Amphipods) in the organic enrichment plots at the end of the experiment (Table 1). The decrease of the richness of taxa between the start and the end of the experiment was consistent in each plot but resulted significantly higher in the OMN than in the OM and Control plots (Table 2).

Nematode composition

Sixty nematode genera (belonging to 26 families) and 74 species were identified in the present study. The highest number of families, genera and species was always observed in Control plots (21–18, 36–29, 36–37, at the start and the end of the experiment, respectively). Chromadoridae was the most abundant family at the start of the experiment whereas Selachinematidae, Chromadoridae, Desmodoridae resulted particularly abundant at the end of the experiment (Table 3). Among all of the families encountered at the start of the experiment, Diplopeltidae, Oxystominidae, Rhabdodemaniidae and Tripyloididae disappeared at the end of the experiment in OM and OMN plots, while other families which were absent initially, appeared: Haliplectidae (1.4%), Aegialolaimidae (0.4%) and Aponchidae (0.4%).

Richtersia was the most abundant genus both at the start and at the end of the experiment, but nematode species composition changed during the experiment in all plots. At the start of the experiment, among the most abundant species, *Graphonema sp1*, *Desmoscolex sp1* and *Bolbolaimus sp1* were observed, while *Sabatieria sp1*, *Chromadorella sp1* and *Polysigma sp1* dominated at the end of the experiment in OM and OMN plots (Table 4).

Of the 74 identified species, 25 disappeared during the experiment and 15 new species appeared. SIMPER analysis indicated that the coefficients of dissimilarity between Control and treated plots increased from the start to the end of the experiment. The differences between Control and treated plots increased consistently from the start to the end of the experiment (Table 5). At the end of the experiment, the Cluster analyses based on the nematode species composition revealed the presence of clear differences in nematode species composition between the Control and OM and OMN plots (Fig. 5a, b). Finally the ANOSIM analysis revealed that nematode species composition did not change between the start and the end of the experiment in the Control plots. Conversely, species composition was significantly different from the start to the end of the experiment in the treated plots

Table 1 Output of the two-way analysis of variance on meiofaunal abundance, richness of taxa, nematode diversity as E(51) and the abundance of each nematode trophic group

	Source	DF	MS	F	*P*	SNK(Time)			SNK(Treatment)	
						Control	OM	OMN	Start	End
Meiofaunal abundance	Time (TI)	1	23.12	46.25	***	End < Start	End < Start	End < Start		
	Treatement(TR)	2	6.49	12.98	**				ns	[OM, OMN] < Control
	TI × TR	2	0.62	1.25	ns					
	Residuals	12	0.50							
	Total	17								
Number of taxa	Time (TI)	1	29.39	43.97	***	End < Start	End < Start	ns		
	Treatement (TR)	2	3.39	5.07	**				Control > OM > OMN	ns
	TI × TR	2	5.06	7.56	**					
	Residuals	12	0.67							
	Total	17								
Nematode diversity E(51)	Time (TI)	1	0.01	0.00	ns	na	na	na		
	Treatement (TR)	2	12.95	0.67	ns				na	na
	TI × TR	2	4.56	0.24	ns					
	Residuals	12	19.28							
	Total	17								
Abundance of 1A	Time (TI)	1	1027.56	9.39	**	End < Start	End < Start	End < Start		
	Treatement (TR)	2	120.27	1.10	ns				na	na
	TI × TR	2	121.89	1.11	ns					
	Residuals	12	109.39							
	Total	17								

Table 1 continued

	Source	DF	MS	F	*P*	SNK(Time)			SNK(Treatment)	
						Control	OM	OMN	Start	End
Abundance of 1B	Time (TI)	1	9.68	0.09	ns	na	na	na		
	Treatement (TR)	2	243.64	2.29	ns				na	na
	TI × TR	2	240.91	2.27	ns					
	Residuals	12	106.22							
	Total	17								
Abundance of 2A	Time (TI)	1	649.20	6.18	**	ns	ns	End < Start		
	Treatement (TR)	2	427.03	4.07	*				ns	[OM, OMN] < Control
	TI × TR	2	130.67	1.24	ns					
	Residuals	12	104.99							
	Total	17								
Abundance of 2B	Time (TI)	1	698.13	18.65	**	ns	End < Start	End < Start		
	Treatement (TR)	2	32.50	0.87	*				ns	[OM, OMN] < Control
	TI × TR	2	38.40	1.03	ns					
	Residuals	12	37.43							
	Total	17								

Output of the Student Newman-Keuls (SNK) post-hoc comparison tests (at $\alpha = 0.05$) carried out to identify when (SNK Time) and among which sets of plots (SNK Treatment) the significant differences occurred. na: not available. ns: not significant. * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$

Table 2 Output of the one-way analysis of variance for the shift from the start to the end of the experiment, normalized to the start of the experiment, for meiofaunal abundance, richness of taxa, nematode diversity as E(51) and the abundance of each nematode trophic group

	Source	DF	MS	F	*P*	SNK (Shift)	Tendency
Meiofaunal abundance	Treatement (TR)	2	0.01	1.21	ns	na	
	Residuals	6	0.01				
	Total	8					
Number of taxa	Treatement (TR)	2	0.18	34.82	***	OMN > [OM, Control]	Decrease
	Residuals	6	0.01				
	Total	8					
Nematode diversity E(51)	Treatement (TR)	2	0.07	0.70	ns	na	
	Residuals	6	0.09				
	Total	8					
Abundance of 1A	Treatement (TR)	2	0.54	91.12	***	[OM, OMN] > Control	Decrease
	Residuals	6	0.01				
	Total	8					
Abundance of 1B	Treatement (TR)	2	1.63	5.76	*	OMN > Control	Increase
	Residuals	6	0.28				
	Total	8					
Abundance of 2A	Treatement (TR)	2	0.32	19.72	**	[OM, OMN] > Control	Increase
	Residuals	6	0.02				
	Total	8					
Abundance of 2B	Treatement (TR)	2	0.18	6.87	**	[OM, OMN] > Control	Decrease
	Residuals	6	0.03				
	Total	8					

A general pattern (tendency) of each variable is also reported from the start to the end of the experiment. na: not available. ns: not significant. * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$

($P < 0.05$ and $P < 0.01$ for OM and OMN, respectively) and when OM and OMN plots were compared with the Control at the end of the experiment (all $P < 0.05$).

Nematode structural and functional biodiversity

All diversity indexes are summarized in Table 6 and did not display significant differences among plots during the experiment (Tables 1 and 2). All diversity indexes, except for Shannon, were higher in the Control than in the treated plots at the start and the end of the experiment.

Deposit feeders (1A + 1B) were the main trophic group (41–44%) for all experimental plots except for the OM at the end of the experiment when epistrate feeders became the dominant group (52%; Fig. 6). The abundance of selective deposit-, epistrate feeders and predators displayed a high variability between the start and the end of the experiment and among the plots (Table 1). The change (shift) during the experiment was higher in the treated plots than in the Control plots, in particular the decreasing of selective deposit feeders and predators and the increasing of not selective deposit- and epistrate-feeders were significantly higher in OM and OMN plots than in the Control. No significant changes (shift) between the start and the end of the experiment was observed for each trophic group in the Control (Table 2).

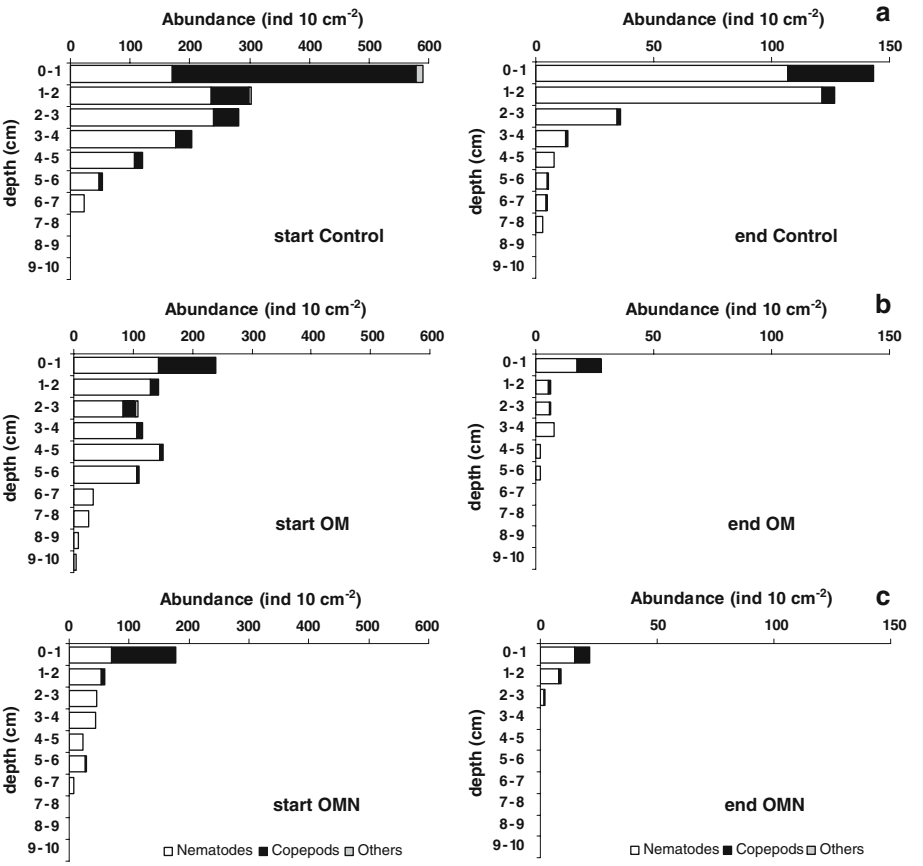

Fig. 4 Meiofaunal vertical profile in the sediments (ind. 10 cm^{-2}). Reported are Nematodes, Copepods (including nauplii) and others higher taxa (including Polychaetes, Ostracods, Tardigrades, Amphipods, Tanaids, Bivalves and *incertae sedis*) at the start and the end of the experiment in the (**a**) Control, (**b**) OM and (**c**) OMN plots, respectively

Discussion

Effect of induced hypoxic conditions on metazoan meiofaunal assemblages

The effects of hypoxic conditions on metazoan meiofaunal assemblages and nematode diversity, inhabiting a dense *P. oceanica* meadow, were investigated by means of in situ experiments. The high spatial heterogeneity of this habitat, and the presence of rizhome and leaves in the corers were responsible for a high variability of meiofaunal assemblages among the selected plots at the start of the experiment. This variability masked the effects of induced hypoxic-anoxic conditions in the experimental plots since, in most cases, different variables displayed the same pattern in the OM, OMN and Control plots (Table 1). The effects of the organic enrichment on the meiofaunal assemblages resulted more evident normalising the shift of all the investigated variables with the value observed at the start of the experiment in each plot (Table 2). This approach allowed unbiased

Table 3 Nematode families and their relative importance in the Control, OM and OMN plots, respectively, at the start and the end of the experiment

Start						End					
Control	%	OM	%	OMN	%	Control	%	OM	%	OMN	%
Chromadoridae	20.7	Chromadoridae	26.8	Chromadoridae	26.4	Selachnematidae	26.0	Desmodoridae	22.2	Chromadoridae	22.7
Desmoscolecidae	14.9	Selachnematidae	17.2	Microlaimidae	13.9	Chromadoridae	22.7	Chromadoridae	15.3	Desmodoridae	21.8
Microlaimidae	12.0	Microlaimidae	14.2	Leptolaimidae	11.4	Desmodoridae	11.9	Comesomatidae	15.3	Comesomatidae	17.3
Xyalidae	11.6	Desmoscolecidae	10.9	Desmoscolecidae	8.2	Desmoscolecidae	10.8	Selachnematidae	12.5	Selachnematidae	11.8
Cyatholaimidae	7.6	Desmodoridae	7.5	Peresianidae	7.9	Microlaimidae	7.1	Desmoscolecidae	5.6	Epsilonematidae	6.4
Desmodoridae	6.9	Oncholaimidae	5.9	Epsilonematidae	7.5	Xyalidae	3.7	Oncholaimidae	5.6	Cyatholaimidae	3.6
Monoposthidae	4.7	Xyalidae	4.2	Selachnematidae	5.7	Oncholaimidae	3.3	Thoracostomopsidae	5.6	Desmoscolecidae	2.7
Oncholaimidae	3.3	Peresianidae	4.2	Desmodoridae	3.9	Thoracostomopsidae	3.0	Psilonematidae	4.2	Oncholaimidae	2.7
Leptolaimidae	2.9	Epsilonematidae	3.3	Xyalidae	3.6	Comesomatidae	2.6	Microlaimidae	4.2	Leptolaimidae	1.8
Comesomatidae	2.9	Thoracostomopsidae	2.1	Monoposthidae	2.5	Leptolaimidae	2.6	Ceramonematidae	2.8	Linhomoeidae	1.8
Peresianidae	2.5	Leptolaimidae	1.3	Oncholaimidae	2.1	Epsilonematidae	2.2	Linhomoeidae	2.8	Microlaimidae	1.8
Thoracostomopsidae	2.2	Comesomatidae	1.3	Thoracostomopsidae	2.1	Cyatholaimidae	1.1	Cyatholaimidae	1.4	Thoracostomopsidae	1.8
Linhomoeidae	2.2	Cyatholaimidae	0.4	Ceramonematidae	1.4	Linhomoeidae	1.1	Haliplectidae	1.4	Axonolaimus	0.9
Epsilonematidae	1.5	Ceramonematidae	0.4	Comesomatidae	1.4	Peresianidae	0.7	Leptolaimidae	1.4	Ceramonematidae	0.9
Oxystominidae	1.1	Axonolaimus	0.4	Linhomoeidae	1.1	Aegialolaimidae	0.4			Haliplectidae	0.9
Selachnematidae	0.7			Enchelidiidae	0.4	Aponchidae	0.4			Peresianidae	0.9

Table 4 Nematode species and their relative importance in the Control, OM and OMN plots, respectively, at the start and the end of the experiment

Start						End					
Control	%	OM	%	OMN	%	Control	%	OM	%	OMN	%
Graphonema sp1	15.3	*Graphonema sp1*	13.6	*Richtersia sp1*	17.2	*Richtersia sp1*	26.0	*Chromadorella sp1*	11.8	*Richtersia sp1*	12.5
Dichro	14.9	*Bolbolaimus sp1*	12.9	*Bolbolaimus sp1*	14.2	*Graphonema sp1*	10.0	*Polysigma sp1*	11.8	*Sabatieria sp1*	12.5
Bolbolaimus sp1	12.0	*Dichromadora sp1*	10.4	*Graphonema sp1*	14.2	*Dichromadora sp1*	9.3	*Richtersia sp1*	11.8	*Polysigma sp1*	11.1
Rynchonema sp1	10.2	*Stephanolaimus sp1*	7.9	*Dichromadora sp1*	12.6	*Desmoscolex sp2*	7.1	*Comesoma sp1*	10.9	*Chromadorella sp1*	9.7
Acanthonchus sp1	7.3	*Epsilonema sp1*	7.5	*Desmoscolex sp1*	10.9	*Bolbolaimus sp1*	5.2	*Graphonema sp1*	8.2	*Desmoscolex sp2*	5.6
Desmodora sp1	6.5	*Leptolaimus sp1*	6.8	*Viscosia sp1*	5.0	*Polysigma sp1*	4.5	*Epsilonema sp1*	6.4	*Viscosia sp1*	5.6
Nudora sp1	4.7	*Desmoscolex sp1*	6.1	*Desmodora sp1*	4.6	*Desmodora sp1*	3.7	*Desmodora sp1*	5.5	*Desmodora sp1*	4.2
Dichromadora sp1	4.4	*Richtersia sp1*	5.7	*Stephanolaimus sp1*	4.2	*Desmoscolex sp1*	3.7	*Sabatieria sp1*	4.5	*Enoplolaimus sp1*	4.2
Comesoma sp1	2.5	*Diodontolaimus sp1*	3.9	*Epsilonema sp1*	3.3	*Rynchonema sp2*	3.3	*Desmoscolex sp2*	2.7	*Epsilonema sp1*	4.2
Leptolaimus sp1	2.5	*Desmodora sp1*	2.5	*Rynchonema sp1*	2.1	*Desmodora sp3*	3.0	*Paracanthonchus sp2*	2.7	*Desmodora sp3*	2.8
Stephanolaimus sp1	2.5	*Nudora sp1*	2.5	*Rynchonema sp2*	2.1	*Viscosia sp1*	2.6	*Viscosia sp1*	2.7	*Graphonema sp1*	2.8
Viscosia sp1	2.5	*Desmoscolex sp2*	2.1	*Enoplolaimus sp1*	1.7	*Enoplolaimus sp1*	2.2	*Desmodora sp2*	1.8	*Metalinhomoeus sp1*	2.8
Enoplolaimus sp1	1.8	*Rynchonema sp1*	2.1	*Polysigma sp1*	1.7	*Epsilonema sp1*	2.2	*Desmodora sp3*	1.8	*Microlaimus sp1*	2.8
Epsilonema sp1	1.5	*Viscosia sp1*	2.1	*Desmodora sp2*	1.3	*Microlaimus sp1*	1.9	*Dichromadora sp1*	1.8	*Polysigma sp2*	2.8
Megadesmolaimus sp1	1.5	*Enoplolaimus sp1*	1.8	*Oncholaimellus sp1*	0.8	*Sabatieria sp1*	1.9	*Diodontolaimus sp1*	1.8	*Prochromadorella sp1*	2.8
Halalaimus isaitshikavi	1.1	*Graphonema sp2*	1.4	*Comesoma sp1*	0.4	*Graphonema sp2*	1.1	*Enoplolaimus sp1*	1.8	*Pselionema sp1*	2.8
Elzalia sp1	0.7	*Sabatieria sp1*	1.4	*Diodontolaimus sp1*	0.4	*Onchium sp1*	1.1	*Microlaimus sp1*	1.8	*Bolbolaimus sp1*	1.4
Oncholaimellus sp1	0.7	*Metachromadora sp1*	1.1	*Enoploides sp1*	0.4	*Paralinhomoeus sp1*	1.1	*Acanthonchus sp1*	0.9	*Diodontolaimus sp1*	1.4
Richtersia sp1	0.7	*Microlaimus sp1*	1.1	*Leptolaimus sp1*	0.4	*Rhips sp1*	1.1	*Graphonema sp2*	0.9	*Enoploides sp1*	1.4
Southerniella sp1	0.7	*Paralinhomoeus sp1*	1.1	*Leptolaimus sp2*	0.4	*Rhips sp2*	1.1	*Metalinhomoeus sp1*	0.9	*Paracyatholaimodes sp1*	1.4
Actinonema sp1	0.4	*Pselionema sp1*	1.1	*Metacyatholaimus sp1*	0.4	*Diodontolaimus sp1*	0.7	*Odontophora sp1*	0.9	*Pierrickia sp1*	1.4

Table 4 continued

Start						End					
Control	%	OM	%	OMN	%	Control	%	OM	%	OMN	%
Chromaspirina sp1	0.4	*Euchromadora sp1*	0.7	*Odontophora sp1*	0.4	*Oncholaimellus sp1*	0.7	*Paralinhomoeus sp1*	0.9	*Pierrickia sp2*	1.4
Deontolaimus sp1	0.4	*Rynchonema sp2*	0.7	*Paramesonchium sp2*	0.4	*Paracanthonchus sp2*	0.7	*Pierrickia sp2*	0.9	*Polysigma sp3*	1.4
Draconema sp1	0.4	*Chromadora sp1*	0.4	*Pselionema sp1*	0.4	*Stephanolaimus sp1*	0.7	*Polysigma sp3*	0.9	*Setoplectus sp1*	1.4
Gairleanema sp1	0.4	*Enoploides sp1*	0.4	*Vasosotoma sp1*	0.4	*Cyartonema sp1*	0.4	*Pselionema sp1*	0.9		
Linhystera sp1	0.4	*Leptolaimus sp2*	0.4			*Desmodora sp2*	0.4	*Sabatieria sp2*	0.9		
Mesacanthoides sp1	0.4	*Metadasynemoides sp1*	0.4			*Enoploides sp1*	0.4	*Setoplectus sp1*	0.9		
Metalinhomoeus sp1	0.4	*Onchium sp1*	0.4			*Epacantion sp1*	0.4	*Stephanolaimus sp1*	0.9		
Metoncholaimus sp1	0.4	*Paramonhystera sp1*	0.4			*Leptolaimus sp1*	0.4				
Paracanthonchus sp1	0.4	*Pareurystomina sp1*	0.4			*Leptolaimus sp2*	0.4				
Paramesonchium sp1	0.4	*Polysigma sp1*	0.4			*Metachromadora sp1*	0.4				
Pselionema sp1	0.4	*Rhabdodemania sp1*	0.4			*Metacyatholaimus sp1*	0.4				
Rhabdodemania sp1	0.4	*Valvaelaimus sp1*	0.4			*Nudora sp1*	0.4				
Rhips sp1	0.4					*Pierrickia sp1*	0.4				
Spilophorella sp1	0.4					*Rynchonema sp1*	0.4				
Xyala sp1	0.4					*Sabatieria sp2*	0.4				
						Synonema sp1	0.4				

Table 5 Coefficients of dissimilarity in nematode species composition (data abundance presence/absence transformed) between OM-OMN and Control plots

Plot	% coefficient of dissimilarity	
	Start	End
Control vs OM	53	56
Control vs OMN	47	66

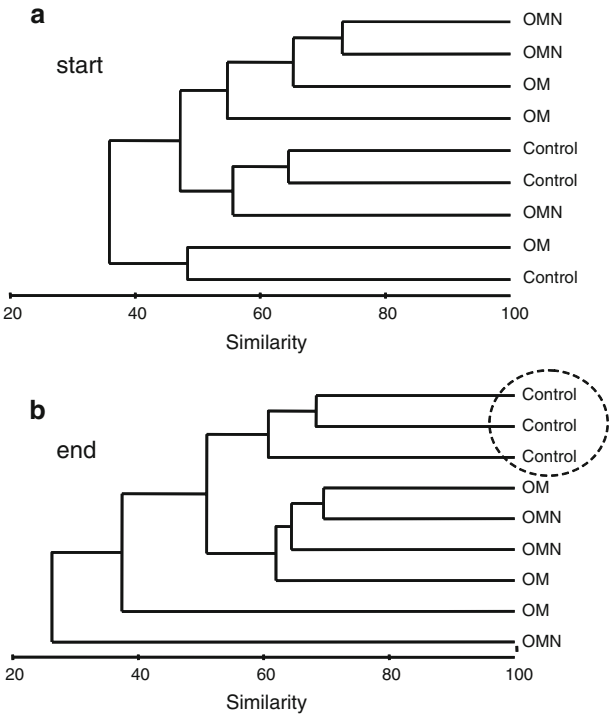

Fig. 5 Cluster analyses based on the species composition in the Control, OM and OMN plots at (**a**) the start and (**b**) the end of the experiment, respectively

interpretations of the effects of the induced hypoxic-anoxic conditions on meiofaunal assemblages inhibiting *P. oceanica* meadows.

The addition of sugar and nutrients in a pristine area resulted in immediate changes in sediment characteristics as reported in details in Pérez et al. (2007). Sediments enriched with organic sources (i.e., OM and OMN plots) became rapidly anoxic, black and rich in sulphides as reported after three months from the start of the experiment (intermediate sampling for sulphur pools, May 2007; Pérez et al. 2007). No evident changes were observed in the sediments of the Control plots, which were characterised by low sulphide concentrations.

The hypoxic-anoxic conditions of OM and OMN plots (Pérez et al. 2007), resulted in the significant decrease of metazoan meiofaunal richness of higher taxa but, apparently, no effects were observed on meiofaunal abundance. According to previous studies dealing with the effects of the organic enrichment on meiofauna (Modig and Olafsson 1998; Schratzberger and Warwick 1998; Mazzola et al. 1999; -2000; La Rosa et al. 2001; Austen and Widdicombe 2006), such changes are due to the reduced oxygen availability and toxic

Table 6 Diversity indexes. Reported are: SR (Species Richness); D (Margalef index); H' (Shannon index), ES(51), J (Evenness) and ITD (Index of Trophic Diversity), in the Control, OM and OMN plots, respectively, at the start (S) and the end (E) of the experiment

Plot	SR		D		H′		E(51)		J		ITD	
	S	E	S	E	S	E	S	E	S	E	S	E
Control	36	37	4.1 ± 1.1	4.8 ± 0.6	3.6 ± 0.3	3.7 ± 0.3	15.3 ± 3.2	17.4 ± 1.3	0.845 ± 0.024	0.813 ± 0.062	0.280	0.310
OM	33	28	4.1 ± 0.6	4.0 ± 1.1	3.6 ± 0.1	3.5 ± 0.5	15.3 ± 0.9	14.1 ± 5.9	0.825 ± 0.037	0.918 ± 0.047	0.300	0.390
OMN	25	24	3.5 ± 0.7	3.7 ± 1.9	3.3 ± 0.4	3.2 ± 1.2	13.9 ± 2.9	13.0 ± 7.8	0.826 ± 0.057	0.930 ± 0.026	0.270	0.310

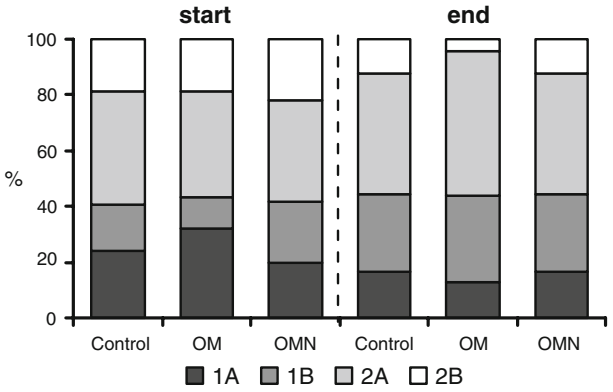

Fig. 6 Trophic structure of nematode assemblages in the Control, OM and OMN plots at the start and the end of the experiment. Reported are the percentages of 1A selective deposit feeders, 1B non-selective deposit feeders, 2A epistrate feeders and 2B predators/ omnivores

effect of the high H_2S concentrations induced by the organic enrichment. This was also confirmed by the high shoot mortality and the reduction of biomass of the *P. oceanica* meadow as a consequences of the direct toxic effects of sulphide intrusion to the plants compared to the Control plots (Pérez et al. 2007). At the start of the experiment, meiofaunal abundance showed high variability among the different types of plots and among replicates within the same plot (i.e. in OM plot, the value of abundance of one replicate was 3–5 times higher than the other two replicates, Fig. 3). This high variability can be related to the complexity of the substrates (sediments, rizhome and root parts) as strongly influenced the distribution of meiofaunal organisms in the *P. oceanica* seagrass and masked the potential effects of the hypoxic-anoxic conditions on meiofaunal abundance. This high variability is reported in previous studies on meiofaunal assemblages in a *P. oceanica* meadow where meiofaunal abundance displayed high variability in each sampling performed on monthly basis (Danovaro and Gambi 2002). In the present experiment, the different plots were situated 5 m apart to avoid any influence of the organic enrichment on the Control plots as suggested by the low concentrations of sulphur pools reported in the intermediate and final sampling (Pérez et al. 2007). Meiofaunal abundances observed in Control plots were comparable to those reported in other *P. oceanica* beds of the NW Mediterranean where values displayed evident temporal variability (Ligurian Sea; Danovaro 1996; Danovaro and Gambi 2002). Both nematodes and copepods are most likely characterised by strong temporal variability also on monthly basis (Danovaro and Gambi 2002) and we can conclude that the differences reported in the Control plots are related to the temporal variability of the meiofaunal assemblages since no changes in sulphide pools were observed in the sediments. Hypoxic conditions altered meiofaunal community structure: nematodes, the most tolerant taxon to the hypoxia, increased their dominance at the end of the experiment as a consequence of the reduction of copepods abundance and the complete disappearance of the other higher taxa (with the exception of few amphipods in the OMN plots). Nematodes are the main components of the "thiobios", which is composed by organisms adapted to live, temporarily, in anoxic sediments (Powell 1989; Giere 1993; Modig and Olafsson 1998). Despite their numerical decrease, some copepods were still present in OM and OMN plots at the end of the experiment, thus suggesting that a) some copepod species are tolerant to the effects of organic enrichment or/and b) are very recent and temporary invaders of the experimental plots and these aspects require further investigations. However, our results are consistent with those reported by Mazzola et al. (2000) in sediment beneath fish farms and by Sandulli and De Nicola-Giudici 1990, 1991 in sediments close to a sewage discharge.

Additional evidence of the effects of the organic enrichment is provided by the analysis the vertical profile of metazoan meiofaunal distribution in the sediment. The high sulphide concentrations in deeper sediment layers of both OM and OMN plots hampered meiofaunal penetration below 5–6 cm and 2–3 cm depth, respectively. Conversely, in the Control and in the OMN and OM plots at the start of the experiment, metazoan meiofauna were able to penetrate up to 10 cm depth as typically observed in pristine coastal sediments (Danovaro et al. 2000a; Vanaverbeke et al. 2004).

Effects of hypoxic-anoxic conditions on nematode diversity

The analysis of nematodes to species level provided useful insights on the impact of organic enrichment and induced hypoxic-anoxic conditions in the seagrass sediments. Conversely to what was expected, the values of species richness were comparable among the different sets of plots and no significant differences were reported. Our results suggest that hypoxic-anoxic conditions did not influence the number of species within nematode assemblages. However, the induced anoxia provoked evident changes in the nematode community with the increase of the coefficient of dissimilarity in the species composition (i.e. species turnover). In fact in the Control plots nematode assemblages displayed low variability in the species composition during the experiment whereas the plots enriched with organic matter displayed significant changes in the nematode species composition from the start to the end of the experiment. Therefore, while at the start of the experiment the nematode community of Control, OM and OMN plots were mixed up, the hypoxic-anoxic conditions increased the dissimilarity of species composition between OM and OMN plots and the Control (Fig. 5 a,b). This suggests that nematode assemblages are subjected to temporal variability in the species composition (as observed in the Control plots) but the induced hypoxic conditions increased the dissimilarity a) within OM and OMN plots between the start and the end of the experiment; b) among the OM, OMN and Control plots at the end of the experiment. Moreover, our results suggest that the highest turnover diversity was present within the OMN plots. The high concentrations of sulphur pools increased the dissimilarity in species composition as suggested by the analysis of the turnover diversity between OM-OMN vs. Control plots (Table 5). The increase in turnover diversity was due partly to a species replacement and partly to changes in the relative importance in the species consistently present in each treated plots. In fact, the most abundant genera at the start of the experiment, *Desmoscolex* and *Bolbolaimus*, disappeared in treated plots, thus revealing a highly sensitivity to the hypoxic-anoxic condition. These genera were replaced by *Chromadorella*, *Sabatiera* and *Polysigma*, which resulted more tolerant to these conditions.

Our results indicate that *Sabatiera,* being abundant in hypoxic-anoxic sediments, (3–6 times more abundant in the treated plots than in the Control) could be proposed as indicator of organic enrichment (Vanreusel 1990; Vincx et al. 1990; Lampadariou et al. 1997; Schratzberger and Warwick 1998; Mirto et al. 2002; Schratzberger et al. 2007; Steyaert et al. 2007) and suggest a species-specific response to the effects of these extreme conditions (Essink and Keidel 1998; Bongers and Ferris 1999; Mirto et al. 2002; Vanaverbeke et al. 2004; Steyaert et al. 2007).

Despite of the relevant changes in the nematode species composition in hypoxic plots, all other diversity indexes did not display significant differences and we observed an increase of the nematode species evenness in OM and OMN plots at the end of the experiment. These results were surprising since ecological disturbance generally determine

a reduction of diversity and of the evenness (Steyaer et al. 2007). These suggest that changes induced by the presence of hypoxic-anoxic conditions in a *P. oceanica* meadow had a minor impact in terms of diversity indexes, but had important effects on the community structure and species composition of meiofaunal assemblages.

The high species turnover and the significant change of the nematode functional diversity (as trophic diversity) observed in this experiment can have important ecological implications on the functioning of seagrass habitats, since nematodes are a food source for higher trophic levels and, in particular, for juvenile fishes associated to these ecosystems. We also found that in treated plots, non-selective deposit- and epistrate-feeders increased significantly from the start to the end of the experiment whilst the contribution of selective deposit feeders and predators significantly decreased in the OM and OMN plots. Since meiofaunal predators can alter macrofaunal community structure, by predating selectively certain species of the temporary meiofauna (Danovaro et al. 1995), the changes of the nematode trophic composition induced by the hypoxic-anoxic conditions might have important consequences on macrofaunal assemblages and higher trophic levels (Danovaro et al. 1995b; Amara et al. 2000).

Conclusions

Our results based on in situ experiments indicate that meiofaunal assemblages changed significantly in response to the hypoxic-anoxic conditions, with an immediate reduction of the richness of higher taxa. Nematodes were the most tolerant organisms as, conversely to what was expected, their species richness did not change in plots characterised by high sulphur pools. Their species composition displayed significant changes between the start and the end of the experiment in the treated plots and among the Control, OM and OMN plots at the end of the experiment. Our results suggest that natural or anthropogenic phenomena inducing hypoxic-anoxic conditions in seagrass systems, can alter the nematode structural and functional biodiversity with potentially important implications on benthic food webs of these ecosystems.

Acknowledgements This work has been carried out within the frame of the project MedVeg (Q5RS-2001-02456). The authors thank Italian research grants FIRB 2001 (RBAU 012 KXA_009) funded by MIUR. This work is a contribution to the NoE MARBEF, financially supported by the EU. The authors are indebted to Antonio Pusceddu for support in the statistical analyses.

References

Albertelli G, Covazzi-Harriague A, Danovaro R et al (1999) Differential responses of bacteria, meiofauna and macrofauna in a shelf area (Ligurian Sea, NW Mediterranean): role of food availability. J Sea Res 42:11–26. doi:10.1016/S1385-1101(99)00012-X

Amara R, Lagardere F, Desaunay Y et al (2000) Metamorphosis and estuarine colonization in the common sole *Sole solea* (L.): implications for recruitment regulation. Oceanol Acta 23:469–484. doi:10.1016/S0399-1784(00)00134-1

Austen MC, Widdicombe S (2006) Comparison of the response of meio- and macrobenthos to disturbance and organic enrichment. J Exp Mar Biol Ecol 330:96–104. doi:10.1016/j.jembe.2005.12.019

Bongers T, Ferris H (1999) Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol Evol 14(6):224–228. doi:10.1016/S0169-5347(98)01583-3

Brown JR, Gowen RJ, McLusky DS (1987) The effect of salmon farming on the benthos of a Scottish sea Loch. J Exp Mar Biol Ecol 109:39–51. doi:10.1016/0022-0981(87)90184-5

Clarke KR (1993) Non parametric multivariate analysis of changes in community structure. Aust J Ecol 18:117–143. doi:10.1111/j.1442-9993.1993.tb00438.x
Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Mar Ecol Prog Ser 210:223–253. doi:10.3354/meps210223
Coull BC (1988) Chapter 3 ecology of the marine meiofauna introduction to the study of meiofauna. Smithsonian Institution Press, Washington, DC London, pp 18–38
Danovaro R (1996) Detritus-Bactera-Meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterrraenan. Mar Biol (Berl) 127:1–13. doi:10.1007/BF00993638
Danovaro R (2003) Pollution threats in Mediterranean Sea: an overview. Chem Ecol 19:15–32. doi:10.1080/0275754031000081467
Danovaro R, Fabiano M (1997) Seasonal Changes in Quality and Quantity of Food Available for Benthic Suspension-feeders in the Golfo Marconi (North-western Mediterranean). Estuar Coast Shelf Sci 44:723–736. doi:10.1006/ecss.1996.0135
Danovaro R, Gambi C (2002) Biodiversity and trophic structure of nematode assemblages in seagrass systems: evidence for a coupling with changes in food availability. Mar Biol (Berl) 141:667–677. doi:10.1007/s00227-002-0857-y
Danovaro R, Della Croce N, Eleftheriou A et al (1995a) Meiofauna of the deep Eastern Mediterranean Sea: distribution and abundance in relation to bacterial biomass, organic matter composition and other environmental factors. Prog Oceanogr 36:329–341. doi:10.1016/0079-6611(96)00002-X
Danovaro R, Fraschetti S, Belgrano A et al (1995b) The potential impact of meiofauna on the recruitment of macrobenthos in a subtidal coastal benthic community of the Ligurian Sea: a field result. In: Eleftheriou A, Ansell AD, Smith CJ (eds) Biology and ecology of shallow coastal waters. Olsen and Olsen, Fredensborg, Denmark, pp 115–122
Danovaro R, Gambi C, Manini E et al (2000a) Meiofauna response to a dynamic river plume front. Mar Biol (Berl) 137:359–370. doi:10.1007/s002270000353
Danovaro R, Tselepides A, Otegui A et al (2000b) Dynamics of meiofaunal assemblages on the continental shelf and deep-sea sediments of the Cretan Sea (NE Mediterranean): relationships with seasonal changes in food supply. Prog Oceanogr 46:367–400. doi:10.1016/S0079-6611(00)00026-4
Danovaro R, Gambi C, Luna G et al (2004) Sustainable impact of mussel farming in the Adriatic Sea (Mediterranean Sea): evidence form biochemical, microbial and meiofaunal indicators. Mar Pollut Bull 49:325–333. doi:10.1016/j.marpolbul.2004.02.038
De Troch M, Van Gansbeke D, Vincx M (2006) Resource availability and meiofauna in sediment of tropical seagrass beds: local versus global trends. Mar Environ Res 61:59–73. doi:10.1016/j.marenvres.2005.05.003
Deprez T et al (2005) NeMys. World wide web electronic publication. www.nemys.ugent.be, version (1/2007)
Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioral responses of benthic macrofauna. Oceanogr Mar Biol Annu Rev 33:245–303
Essink K, Keidel H (1998) Changes in estuarine nematode communities following a decrease of organic pollution. Aquat Ecol 32:195–202. doi:10.1023/A:1009901207217
Fossing H, Jørgensen BB (1989) Measurement of bacterial sulfate reduction in sediments: evaluation of the single-step chromium reduction method. Biogeochemistry 8:205–222. doi:10.1007/BF00002889
Fraschetti S, Gambi C, Giangrande A et al (2006) Structural and functional response of meiofauna rocky assemblages to sewage pollution. Mar Pollut Bull 52:540–548. doi:10.1016/j.marpolbul.2005.10.001
Frederiksen MS, Holmer M, Pérez M, Invers O, Ruiz JM, Knudsen BB (2008) Effect of increased sediment sulfide concentrations on the composition of stable sulfur isotopes (δ 34S) and sulfur accumulation in the seagrasses *Zostera marina* and *Posidonia oceanic*. J Exp Mar Biol Ecol (in press)
Gambi C, Vanreusel A, Danovaro R (2003) Biodiversity of nematode assemblages from deep-sea sediments of the Atacama Slope and Trench (South Pacific Ocean). Deep Sea Res Part I Oceanogr Res Pap 50:103–117. doi:10.1016/S0967-0637(02)00143-7
Giere O (1993) Meiobenthology. Springer-Verlag, Germany
Grall J, Chauvaud L (2002) Marine eutrophication and benthos: the need for new approaches and concepts. Glob Change Biol 8:813–830. doi:10.1046/j.1365-2486.2002.00519.x
Gray JS (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. J Exp Mar Biol Ecol 250:23–49. doi:10.1016/S0022-0981(00)00178-7
Gray JS, Wu RSS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. Mar Ecol Prog Ser 238:249–279. doi:10.3354/meps238249
Green EP, Short FT (2003) World atlas of seagrasses. Prepared by the UNEP World Conservation Monitoring Centre, University of California Press, Berkeley, USA
Hansen BW, Stenalt E, Petersen JK et al (2002) Invertebrate re-colonisation in Mariager Fjord (Denmark) after severe hypoxia. I. Zooplankton and settlement. Ophelia 56:197–213

Heip C, Vincx M, Vranken G (1985) The ecology of marine nematodes. Oceanogr Mar Biol Annu Rev 23:399–489
Hendelberg M, Jensen P (1993) Vertical distribution of the nematode fauna in a coastal sediment influenced by seasonal hypoxia in the bottom water. Ophelia 37:83–94
Holmer M, Frederiksen M, Møllegaard H (2005) Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. Aquat Bot 81:367–379. doi:10.1016/j.aquabot.2004.12.006
Hulbert SH (1971) The non-concept of species diversity: a critique and alternative parameters. Ecology 52:577–586. doi:10.2307/1934145
Jørgensen BB, Richardson K (1996) Eutrophication in coastal marine ecosystems. Coastal and estuaries studies 52. American Geophysical Union, Washington DC, p 267
Josefson AB, Widbom B (1988) Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar Fjord basin. Mar Biol (Berl) 100:31–40. doi:10.1007/BF00392952
Karakassis I, Tsapakis M, Hatziyanni E et al (2000) Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES J Mar Sci 5(5):1462–1471. doi:10.1006/jmsc.2000.0925
Koroleff F (1983) Determination of ammonia. In: Grasshoff K, Ehrhardt M, Kremling K (eds) Methods of seawater analysis: second (Revised and extended edn). Verlag Chemie, Weinheim, pp 150–157
Kristensen E (2000) Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1–24. doi:10.1023/A:1003980226194
La Rosa T, Mirto S, Mazzola A et al (2001) Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. Environ Pollut 112:427–434. doi:10.1016/S0269-7491(00)00141-X
Lampadariou N, Austen MC, Robertson N et al (1997) Analysis of meiobenthic community structure in relation to pollution and disturbance in Iraklion Harbour, Greece. Vie Milieu 47:9–24
Leguerrier D, Niquil N, Boileau N et al (2003) Numerical analysis of the food web of an intertidal mudflat ecosystem on the Atlantic coast of France. Mar Ecol Prog Ser 246:17–37. doi:10.3354/meps246017
Livingston RJ (2001) Eutrophication processes in coastal systems: origin and succession of plankton blooms and effects on secondary production in Gulf Coast estuaries. CRC Press, New York
Margalef DR (1958) Information theory in ecology. Gen Syst 3:36–71
Mazzola A, Mirto S, Danovaro R (1999) Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the Western Mediterranean. Mar Pollut Bull 38(12):1126–1133. doi:10.1016/S0025-326X(99)00142-3
Mazzola A, Mirto S, La Rosa T et al (2000) Fish-farming effects on benthic community structure in coastal sediments: analysis of meiofaunal recovery. ICES J Mar Sci 57:1454–1461. doi:10.1006/jmsc.2000.0904
Meyers MB, Fossing H, Powell EN (1987) Microdistributions of interstitial meiofauna, oxygen and sulphide gradients, and the tubes of macro-infauna. Mar Ecol Prog Ser 35:223–241. doi:10.3354/meps035223
Mirto S, La Rosa T, Gambi C et al (2002) Nematode community response to fish-farm impact in the Western Mediterranean. Environ Pollut 116:203–214. doi:10.1016/S0269-7491(01)00140-3
Modig H, Olafsson E (1998) Responses of Baltic benthic invertebrates to hypoxic events. J Exp Mar Biol Ecol 229:133–148. doi:10.1016/S0022-0981(98)00043-4
Murell MC, Fleeger JW (1989) Meiofauna abundance on the Gulf of Mexico continental shelf affected by hypoxia. Cont Shelf Res 9:1049–1062. doi:10.1016/0278-4343(89)90057-5
Nagelkerken I, van der Velde G, Gorissen MW et al (2000) Importance of mangroves, seagrass beds and shallow coral reefs as nursery for important coral reef fishes, using a visual census techniques. Estuar Coast Shelf Sci 51:31–44. doi:10.1006/ecss.2000.0617
Nixon SW (1995) Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199–219
Nordberg K, Filipsson HL, Gustafsson M et al (2001) Climate, hydrographic variations and marine benthic hypoxia in Koljo Fjord, Sweden. J Sea Res 46:187–200. doi:10.1016/S1385-1101(01)00084-3
Painting SJ, Devlin MJ, Malcolm SJ et al (2007) Assessing the impact of nutrient enrichment in estuaries: susceptibility to eutrophication. Mar Pollut Bull 55:74–90. doi:10.1016/j.marpolbul.2006.08.020
Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr Mar Biol Annu Rev 16:229–311
Penhale PA, Wetzel RG (1983) Structural and functional adaptations of eelgrass (*Zostera marina L.*) to the anaerobic sediment environment. Can J Bot 61:1421–1428
Pérez M, Invers O, Ruiz JM et al (2007) Physiological responses of the seagrass *Posidonia oceanica* to elevated organic matter content in the sediments: an experimental assessment. J Exp Mar Biol Ecol 344:149–160. doi:10.1016/j.jembe.2006.12.020
Peterson CH, Summerson CH, Thomson E et al (2000) Synthesis of linkages between benthic and fish communities as a key to protecting essential fish habitat. Bull Mar Sci 66:759–744

Pielou EC (1975) Ecological diversity. Wiley and Sons, New York, p 165
Platt HM, Warwick RM (1983) Free living marine nematodes. Part I British enoplids. Linnean Society of London and The Estuarine Brakish-water Sciences Association by Cambridge University Press, pp 1–306
Platt HM, Warwick RM (1988) Free living marine nematodes. Part II British chromadorids. Linnean Society of London and The Estuarine Brakish-water Sciences Association by Cambridge University Press, pp 1–502
Pocklington P, Scott DB, Schaffer CT (1994) Polychaete response to different aquaculture activities. In: Dauvin JC, Laubier L, Reish DJ (eds) Actes de la 4ème Conférences Internationale des Polychètes. Memoires du Museum National d'histoire Naturelle (France) 162:511–520
Powell EN (1989) Oxygen, sulphide and diffusion: why thiobiotic meiofauna must be sulphide-insensitive first-order respirers. J Mar Res 47:887–932
Powers SP, Peterson CH, Christian RR et al (2005) Effects of eutrophication on bottom habitat and prey resources of demersal fishes. Mar Ecol Prog Ser 302:233–243. doi:10.3354/meps302233
Sanders HL (1968) Marine benthic diversity: a comparative study. Am Nat 102:243–282. doi:10.1086/282541
Sandulli R, de Nicola-Giudici M (1990) Pollution effects on the structure of meiofaunal communities in the bay of Naples. Mar Pollut Bull 21:144–153. doi:10.1016/0025-326X(90)90550-R
Sandulli R, de Nicola-Giudici M (1991) Responses of meiobenthic communities along a gradient of sewage pollution. Mar Pollut Bull 22:463–467. doi:10.1016/0025-326X(91)90217-G
Schratzberger M, Warwick RM (1998) Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. Mar Ecol Prog Ser 164:83–94. doi:10.3354/meps164083
Schratzberger M, Warr K, Rogers SI (2007) Functional diversity of nematode communities in the south-western North Sea. Mar Environ Res 63:368–389. doi:10.1016/j.marenvres.2006.10.006
Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to unhydrous glycerine. Nematologica 4:67–69
Shannon CE, Weaver W (1963) The mathematical theory of communication. University of Illinois Press, Urbana, Illinois, p 111
Somerfield PJ, Rees HL, Warwick RM (1995) Interrelationships in community structure between shallow water marine meiofauna and macrofauna in relation to dredging disposal. Mar Ecol Prog Ser 127:103–112. doi:10.3354/meps127103
Steyaert M, Moodley L, Nadong T et al (2007) Responses of intertidal nematodes to short-term anoxic events. J Exp Mar Biol Ecol 345(2):175–184. doi:10.1016/j.jembe.2007.03.001
Terrados J, Duarte CM, Kamp-Nielsen L et al (1999) Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquat Bot 65:175–197. doi:10.1016/S0304-3770(99)00039-X
Tutsumi H, Kikuchi T, Tanaka M et al (1991) Benthic faunal succession in a cove organically polluted by fish farming. Mar Pollut Bull 23:233–238. doi:10.1016/0025-326X(91)90680-Q
Vanaverbeke J, Steyaert M, Soetaert K et al (2004) Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the southern North Sea. J Sea Res 52:281–292. doi:10.1016/j.seares.2004.02.004
Vanreusel A (1990) Ecology of the free-living marine nematodes from the Voordelta (Southern Bight of the North Sea).I Species composition and structure of the nematode communities. Cah Biol Mar 31:439–462
Vincx M, Meire P, Heip C (1990) The distribution of nematode communities in the Southern Bight of the North Sea. Cah Biol Mar 31:107–129
Vopel K, Dehmlow J, Artl G (1996) Vertical distribution of *Cletocamptus confluens* (Copepoda, Harpacticoida) in relation to oxygen and sulphide microprofiles of a brackish water sulphuretum. Mar Ecol Prog Ser 141:129–137. doi:10.3354/meps141129
Warwick RM, Platt HM, Somerfield PJ (1998) Free living marine nematodes. Part III British monysterids. Linnean Society of London and The Estuarine Brakish-water Sciences Association by Cambridge University Press, pp 1–296
Widdicombe S, Austen MC (2001) The interaction between physical disturbance and organic enrichment: an important element in structuring benthic communities. Limnol Oceanogr 46:1720–1733
Wieser W (1953) Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Arkiv Zool 2(4):439–484
Wu RSS, Lam KS, MacKay DW et al (1994) Impact of marine fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. Mar Environ Res 38:115–145. doi:10.1016/0141-1136(94)90004-3