Response of estuarine free-living nematode assemblages to organic enrichment

Kandratavicius, Noelia ; de Ward, Catalina Pastor ; Venturini, Natalia ; Gimenez Noya, Jose; Rodriguez, Marcel ; Muniz, Pablo

Marine Ecology Progress Series

DOI:
10.3354/meps12699

Published: 23/08/2018

Peer reviewed version

Response of estuarine free-living nematode assemblages to organic enrichment: an experimental approach

Noelia Kandratavicius[*1], Catalina Pastor de Ward[2], Natalia Venturini[3], Luis Giménez[4], Marcel Rodriguez[1] & Pablo Muniz[1].

1 Oceanografía & Ecología Marina, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, Universidad de la República (UdelaR), Iguá 4225, Montevideo, 11400, Uruguay

2 Instituto de Diversidad y Evolución Austral (IDEAUs), CCT, CONICET, Bulevar Almirante Brown 2915, Puerto Madryn, Chubut, Argentina

3 Laboratorio de Biogeoquímica Marina, Oceanografía & Ecología Marina, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, UdelaR, Iguá 4225, Montevideo, 11400, Uruguay

4 School of Ocean Sciences, Bangor University, LL59 5AB, Menai Bridge, Anglesey, UK

*nkandra@fcien.edu.uy

Key words: Free-living nematodes, *Spirulina platensis*, eutrophication, Laguna de Rocha, Uruguay

Running page head: Response of nematodes to organic enrichment.

Abstract

Organic enrichment, especially from anthropogenic sources, is one of the current threats to costal-marine biodiversity. Organic enrichment occurs mainly in sheltered soft bottoms, characterized by fine sediments, and results in multiple changes in the benthic habitat, including hypoxia and the increase in the concentration of compounds that are toxic to marine invertebrates. We report on the results of a microcosm-based experiment (duration = 30 days), quantifying the effects of organic enrichment on taxonomic and functional diversity of nematode assemblages from an open/closed coastal lagoon of South America (Rocha Lagoon, Uruguay). In open/close lagoons, the input of organic matter becomes a major disturbance due the limitation in water renewal. Enrichment led to reductions in abundance, richness and trophic diversity of the nematode assemblage. Rapid reductions in total abundance (after 4 days) were registered, while richness decreased only towards the end of the experiment (~30 days). Trophic changes were characterized by loss of predators/omnivores and dominance of selective deposit-feeders and epigrowth-feeders. By contrast, we did not find any selective effect of enrichment

associated to life history traits (i.e. maturity index). Overall, these findings have two important implications for the conservation and monitoring of the health of coastal lagoons: first, monitoring of the nematode assemblages at genus level is sufficient to detect the enrichment effects; second, an index of trophic diversity would be a good indicator of effects of enrichment on natural communities.

Introduction

Nutrient enrichment of marine/estuarine areas can favour algal growth and lead to eutrophication, the occurrence of anoxia and hypoxia, fish-kills (Glasgow & Burkholder 2000), loss or degradation of habitat for benthic organisms and a decrease in the number of fisheries. Eutrophication is considered one of the major stresses for aquatic environments, and it is characterized by excess biomass (Sampou & Oviatt 1991) and accumulation of refractory organic matter. Anthropic activities including agricultural production, industrial and domestic effluents, modify the physicochemical and biological conditions of estuarine systems (Day et al. 1989, Perissinotto et al. 2010). These activities generally intensify the process of eutrophication introducing inorganic nutrients and the consequent increase in algal biomass and primary productivity in the water column (Cloern 2001, Pusceddu et al. 2009).

Organic enrichment is an important ecological process in marine/estuarine sediments (Kelly & Nixon 1984). Organic enrichment occurs more frequently in habitats characterized by fine sediments, low hydrodynamics and low dissolved oxygen concentration (Snelgrove & Butman 1994). Accumulation of organic compounds (labile and refractory) leads to changes in physical, chemical, biological and ecological features of sediments (Cloern 2001) and defines the quality and amount of food resources, and hence affects metabolic processes and mobility, as well as community structure, biodiversity and trophic structure (Grall & Chauvaud 2002). The labile fraction of the organic matter (carbohydrates, lipids and proteins) is easily digested and assimilated by heterotrophic organisms, and is the major energy source for benthic organisms (Ruhl et al. 2008). By contrast, the refractory fraction (e.g. humic and fulvic acids) are degraded more slowly and do not represent a favourable source of nutrition (Joseph et al. 2008).

However, at moderate levels of organic enrichment, benthic animals may show altered behavioral patterns, decreased feeding and reproduction activity, and changes in physiological functions (see reviews by Vernberg 1972, Herreid 1980). At high levels, organic enrichment, through its effects of oxygen levels and the chemical conditions of

the sediment, can produce important changes in communities and benthic food webs. Enrichment leads to reductions in diversity and community shifts, where the original community is replaced by one characterized by species resistant to organic pollution (Pearson & Rosenberg 1978, Hargrave et al. 2008, Venturini et al. 2012). At these levels, enrichment also leads to an impoverishment of the functional structure of the community (Pearson & Rosenberg 1978). Given that organic matter can cause changes at so many levels of biological organization, excessive input of organic matter can be considered a strong stressor (Pearson & Rosenberg 1978, Diaz & Rosenberg 2008).

In spite of the extensive coverage of the impact of organic enrichment on marine/estuarine ecosystems, the effect of the organic matter on the biota of coastal lagoons is not well documented or is underestimated (Kendall et al. 1995, Armenteros et al. 2010). Coastal lagoons common coastal habitats, for instance Mediterranean Sea, the Gulf of Mexico and Atlantic coast of North America as well as the Atlantic coast of South America. Overall, lagoons comprise 13% of coastal regions globally (Bird 1994, Kjerfve 1994, Antony et al. 2009). In coastal lagoons, the input of organic matter becomes a major disturbance because of the limitation in the capacity for water renewal (Urban et al. 2009). Coastal lagoons are considered particularly vulnerable to eutrophication, due to their restricted exchange with the adjacent sea, their shallow nature, and their high productivity. Lagoon eutrophication results from increasing human population densities along the lagoon coastline and from use of fertilizers for agriculture in their surrounding watershed (Cloern 2001).

Here, we quantified the effects of organic enrichment on taxonomic and functional diversity of assemblages of free-living nematodes from Rocha Lagoon (Atlantic coast of Uruguay, South America). The process of eutrophication existing in Rocha lagoon (see "study area" in "material and methods" for details) is representative of the situation being experienced by other coastal systems worldwide (Cloern 2001). We studied the effects of enrichment, through a laboratory experiments, using nematode assemblages as a model system. Laboratory experiments are considered an appropriate approach to study the effect of organic enrichment in marine and estuarine communities (Coull & Chandler 1992). Microcosm experiments enable the establishment of cause-effect relationships (Nilsson et al. 1991) and can be used to determine which organism are indicators of disturbances (Heip et al. 1985, Coull 1988).

Free-living nematodes are excellent organisms for laboratory experiments purposes, due to their small size, short life cycle, quick response to environmental changes and resistance to sediment manipulation (Warwick et al. 1988). Although the manipulation of field sediment leads to a disruption of the interstitial environment, the response of nematodes has been successfully separated from "microcosm effect" in a procedural control in studies of effects of xenobiotics (Austen & McEvoy 1997, Hedfi et al. 2007), sedimentation (Schratzberger et al. 2000) and organic enrichment (Schratzberger & Warwick 1998, Armenteros et al. 2010). Several ecological factors such as habitat type (e.g. sandy beaches, estuaries, etc.), the origin of organic inputs and the intensity of human disturbances had been proved to affect spatial distributional patterns of free-living nematodes (Schratzberger et al., 2008).

We studied the effect of enrichment on taxonomic diversity at the species/genus levels was well as functional diversity, quantified in terms of feeding types and life strategies. We expected that by combining taxonomic and functional diversity we would obtain a better understanding of the structural components and the functioning of the benthic community (Norling et al. 2007). In particular, for nematodes, the relationships between the functional attributes (e.g. trophic responses) and organic matter amount and quality are not well understood yet. Therefore, our experimental approach also offers the possibility to establish the taxonomic and functional responses of nematodes to enrichment. The patterns observed in experimental approaches may contribute to a better understanding and prediction of the patterns observed in the nature. In particular, we, hypothesized that organic enrichment would lead to reductions in taxonomic diversity and an increase in the abundance of nematodes that are tolerant to disturbance. We also expected low trophic diversity, as well as the dominance of organisms with short life-cycles.

Materials and methods

Environmental set up

Experimental surface sediments and experimental nematodes were collected during January 2015 in the south of Laguna de Rocha, Uruguay (34°39'47.42''S, 54°13'47.36''W, see Figure 1 Kandratavicius et al 2015). Rocha Lagoon is a choked type lagoon (Kjerfve & Magill 1989, Conde et al. 2000) with an area of 7304 ha, shallow and

with an intermittently open-closed connection with the Atlantic Ocean. The communication with the ocean take place several times per year, when depth increases and when the sandbar is breached by wave action (Conde & Rodríguez-Gallego 2002).

Among the major ecological problems of Rocha Lagoon is the recent eutrophication, probably caused by land use and the input of domestic effluents (Rodríguez-Gallego et al. 2008). The industrial activity is limited and is mainly stockbreeding but the lagoon receives anthropogenic inputs from the city of Rocha and the Municipal Slaughterhouse (through from Rocha Stream) and has received further inputs in the past from a fish processing plant and agriculture (Arocena et al. 2000). Recently, Pita et al (2017) using sedimentary organic matter and biochemical composition classified Rocha Lagoon as eutrophic.

Kandratavicius et al. (2015) found that meiofauna is dominated by nematodes (63%), copepods (15%) and ostracods (7%). Nematodes were significantly more abundant in summer and in fine sand, which was more common in the inner zones of Rocha lagoon.

Sampling and microcosm set up

Sediment samples and fauna were taken by hand because of the shallow habitats (<1m) in a location known as "old bar" (34°39'47.20''S, 54°13'50.41''W) characterized by fine sediments (69% mud, fine and medium sand), low organic content (~1.32%), 18.9 of salinity (average from summer season) and high temperatures (28 °C) (Giménez et al. 2014, Kandratavicius et al. 2015). Five plastic cores (2.7 cm internal diameter) were taken to 10 cm depth in the sediment for the description of the community structure and three surface sediment samples (1cm depth, approximately 300 g) for the estimation of total organic matter, chlorophyll a and organic biopolymers (total lipids, carbohydrates and proteins). All of these samples were considered as field control.

A key point for the validity of the experimental study is that it should be as homogeneous as possible across experimental units and the effects of treatments must be stronger than the "microcosm effect", i.e. the effect of manipulation of sediments (Austen & McEvoy 1997). We carefully collected approximately 15 1itres of surface sediment to set up the experimental units or microcosms. The fresh sediment collected in the lagoon was transported to the laboratory, stored in two containers with aeration for approximately 24 hours. Thereafter, the sediment was gently homogenized with a spoon and five random

aliquots of sediment were checked for the presence of living nematodes, identified as individuals moving in the sediment. Each microcosms was considered to be an independent experimental unit and consisted in a 250 ml glass beaker with 150 ml of sediment (resulting in a 4 cm layer of sediment) and lagoon water with individual aerator. The microcosms were placed in a lab table and was kept under natural climatic conditions with temperature ranged from 20°C to 25°C and a summer light/dark cycles of temperate regions (about 14 h light per 24 h). In total 77 microcosms were made: 50 microcosms were used to evaluate the response of the nematode communities to organic enrichment and 27 microcosms to evaluate changes in chlorophyll a, total organic matter and biopolymers (Fig. 1).

The treatment of increased organic matter was created by adding the commercial microalgae Spirulina platensis particulate. The biopolymeric composition of S. platensis was protein 60%, carbohydrates 30% and lipids 10%, this composition was similar to the proportions reported from natural populations (Rios et al. 1998).The chlorophyll a (Chl-a) content was 87 ug gss-1. The Chl-a content in field was 10 ug l-1 (Conde et al. 2003) ; considering those results we modified the method of Armenteros et al. (2010) in order to produce three treatments as follows: (1) High level addition to the microcosm of 5g of S. platensis equivalent to 43.5 ug l-1 Chl-a, around four times the field concentration (24 microcosm = 15 to nematodes community analysis + 9 to sediment analysis); (2) Medium level: addition of 2.5g of S. platensis equivalent to 21.75 ug l-1 Chl-a, around twice the field concentration (24 microcosms = 15 to nematodes community analysis + 9 to sediment analysis) and (3) Control without any addition (29 microcosm = 20 to nematodes community analysis + 9 to sediment analysis).

At the beginning of the experiment (time = T_0), five microcosms of the control treatment were used to analyze the structure of nematode community (microcosms are destroyed during the sampling and thus are used only once). At times of 4, 15 and 30 days, five microcosms of each treatment were used to analyze the structure of the nematodes community and three microcosms were used to analyze the organic matter, biopolymers and Chl-a content (Fig. 1). The dissolved oxygen concentration and temperature were measured daily in the water matrix (measurements done with a O_2 microsensor Unisens® OX50 and YSI® multi-parameter respectively).

Sample processing

The content of each microcosm was used to analyze different attributes of sediment. Photosynthetic pigments (Chl-a and phaeopigments) were analyzed according to Lorenzen (1967), modified by Sündback (1983) for sediments. Total organic matter (OM) was analyzed based on Byers et al. (1978) and expressed as percentage (%). Biochemical composition of organic matter was analyzed following the protocols described in Danovaro (2010). Total protein (PRT) analysis was conducted according to Hartree (1972) modified by Rice (1982) to compensate for phenol interference. Total carbohydrates (CHO) were analyzed according to Gerchacov & Hatcher (1972). Total lipids (LIP) were extracted by ultrasonication with a mixture of chloroform: methanol (1:2 v/v) and analyzed following the protocol described in Marsh &Weinstein (1966). Blanks for each analysis were performed with pre-combusted sediment (450°C, 4 hrs.). PRT, CHO and LIP concentrations were expressed as bovine serum albumin, glucose and tripalmitine equivalents, respectively. Protein, carbohydrate and lipid concentrations were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75 µg, respectively (Fabiano & Danovaro 1994). The sum of protein, lipid and carbohydrate carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable estimate of the labile fraction of organic matter (Fabiano et al. 1995) and to classify the trophic status of the sediments. Also, the protein to carbohydrate ratio (PRT : CHO) and the carbohydrate to lipid ratio (CHO : LIP) were calculated and used as indicators of the status of biochemical degradation processes (Galois et al. 2000).

In order to sample nematodes, the content of each microcosm was washed between a 500 µm sieve and 63 µm one using filtered water. To extract the meiofauna from the sediment fraction, retained on the sieve of 63 µm we applied a flotation technique using Ludox HS 40 Coloidal Silica (1.18 g cm^{-3}) and centrifugation (Heip et al. 1985, Vincx 1996). This process was repeated 3 times whereby each time the supernatant Ludox containing the meiofauna organisms was decanted and washed. The final washed and extracted sample was then preserved in 4% formaldehyde, and a small amount of Rose Bengal was added to facilitate the identification. In binocular loupe 100 nematodes were randomly picked out of each microcosm and mounted on glass slides for genus identification under microscope (Somerfield & Warwick, 1996) using pictorial keys (Platt & Warwick 1983, 1988, Warwick et al. 1998).

Before assembly into glass slides, nematodes were placed in a solution of glycerol-ethanol and allowed to evaporate in a desiccator so that the nematodes remained in glycerin, facilitating the observation of their structures.

Structure of nematode assemblages and biological/functional traits

Richness (as number of genera) and abundance of nematodes per genera was determined for each microcosm. Each one was classified according to their life strategy into a scale of coloniser/persister (c-p score: Bongers 1990, Bongers et al. 1991). The scale range is defined from extreme colonisers (cp score = 1) to extreme persisters (c-p score= 5). The maturity index (MI) of the community was calculated using the formula (Bongers et al. 1991):

$$MI = \Sigma\ (v_{(i)} \times f_{(i)})$$

where *v(i)* = the c-p value of genera i and *f(i)* = the relative frequency of the genera i.

Additionally nematode genera were assigned to feeding types according to Wieser's (1953) classification based on the morphology of buccal cavity: selective deposit-feeder (1A), nonselective deposit-feeder (1B), epigrowth feeder (2A) and omnivore/predator (2B). This classification was used to calculate the Index of Trophic Diversity (Heip et al. 1985), calculated as:

$$ITD = \Sigma\Theta^2$$

where ϴ is the percentage contribution of each trophic group according to Wieser (1953). ITD values vary in a range between 0.25 (high trophic diversity: the four groups have a representation of 25%) and 1.0 (low trophic diversity: a single trophic group dominates, 100%).

Data analysis

Multi and univariate techniques were used for data analysis using the software PRIMER 6.0.2 (Clarke & Gorley 2006) and STATISTICA 10.0 from StatSoft. If needed, data were transformed and re-checked to determine if parametric assumptions were applicable. Comparisons to test changes in biota and trophic status of sediment (based on organic matter, chlorophyll a, phaeopigments, and biopolymers) between control groups were done in order to assess the "microcosm effect" (abiotic and biotic changes due experiment artifacts) using a one-way ANOVA with five levels: field control, time 0 (microcosm control), controls at time 4, 15 and 30. If the differences in ANOVA were significant, two comparisons using least square means (t-test) were performed: (1) field *vs.* T_0 to test the

267 "microcosm effect" i.e. the differences between field and the experimental conditions; (2)
268 T_0 *vs.* controls to 4 days, 15 and 30 (C_4, C_{15}, C_{30}) to test the temporal changes in the
269 microcosm controls. The results of these tests allow establishing the validity of the
270 experimental setting.
271 Treatment effects (i.e. among levels of enrichment) were evaluated in first instance for
272 the trophic status of sediment and separately for a possible effect on the trophic response
273 of nematodes (MI and ITI) or structural changes in the assemblage (richness and
274 abundance per genera). Those tests were carried out through a two-way factorial ANOVA
275 where the treatments of organic matter input (control, medium and high) and time (4, 15,
276 30 days) were used as factors. Treatment effects of dissolved oxygen were evaluated with
277 a repeated measures ANOVA, where time was the repeat factor.
278 Responses of multivariate structure of nematode assemblages to treatments were tested
279 with permutation-based analysis of variance, using PERMANOVA (Anderson et al.
280 2008). Data were square root transformed in order to down weigh the contribution of
281 dominant species. Similarity matrices were built using Bray-Curtis index and
282 permutations were on the reduced model; reported p-values are based on Monte Carlo
283 tests. The SIMPER procedure was applied to look for genera which contribute the most
284 to similarity /dissimilarity across treatments and/or times.

285 **Results**

286 **Abiotic component**

287 Validation of the experimental setting

288 On average, Chl-a, phaeopigments and carbohydrates (Figs. 2 and 3) were significantly
289 higher in the control T0 than in the field (one-way ANOVA and t-test: Chl- a: $F_{4,10}$ =
290 23.73 $p < 0.001$, phaeopigments: $F_{4, 10} = 53.95$ $p < 0.001$, carbohydrates: $F_{4, 10} = 5.26$ $p <$
291 0.001, Table 1). These variables had an important increase in T0 with respect to field
292 (Table 2). The percent increase in Chl-a was 78%, while in phaeopigments was 100%
293 and in carbohydrates was 25%.

294 Only the phaeopigments, Chl-a and organic matter increased significantly over time in
295 the controls (Table 1) where values were more than twice double than those found in the
296 field. Proteins to carbohydrates ratio (PRT : CHO) showed values < 1 in Field, T_0 and
297 controls, while in the treatments recorded values were > 1. Carbohydrates to lipids ratio
298 (CHO/LIP) showed values >>1 in Field, T_0, controls and both treatments.

Enrichment

There was a significant and important enrichment in terms of organic matter, Chl- a and phaeopigments (Fig 2, Table 2 and 3). The addition of *S. platensis* at both densities resulted in significant increase in Chl-a and pheopigments, while the maximum levels or organic matter were clear only under high levels of *S. platensis*. The significant increase in phaeopigments occurred progressively from day four to day 30 (post-hoc test: T4 < T15 < T30, Electronic supplement S2).

The addition of *S. platensis* also increased the levels of BPC, lipids and proteins (Fig. 3. Table 2 and 3) while carbohydrate levels were not significantly affected. Protein levels increased progressively (significant interaction: time:treatment, Electronic supplement S2) while those of BPC and lipids were established quickly, especially after addition of *S. platensis* at the high density treatment.

The addition of *S. platensis* resulted in a significant decrease of dissolved oxygen (Table 3, Fig. 4). Its levels reached the limit of hypoxia (2 mg l^{-1}) after ~ 1 day and tended to recover after around10 days; recovery took place more slowly after addition of *S. platensis* at the highest treatment (Fig. 4, Electronic supplement S2).

Overall, our results (increased levels of pigments, organic matter, lipids, proteins and BPC) validated the experimental setting. Hence, in the remainder of this article, we nominate the treatments as "control", "medium level of enrichment" (= addition of 2.5 g of *S. platensis*) and "high level of enrichment" (=addition 5 g of *S. platensis*). In addition, enrichment led to hypoxia.

Responses of the nematode assemblage

A comparison of univariate measures of assemblages between field and control samples at day 0 indicates how much the assemblages in the experimental conditions mimic those of the natural environment (Fig. 5). In general, nematode assemblages were similar in field, control microcosms and T_0. There were no significant differences in the log-transformed number of nematodes ($F_{4,20}$ =1.641 $p > 0.05$). Also, nematodes abundance did not vary significantly at the end of the experiment in comparison with T_0.

Nineteen genera of free-living marine/estuarine nematodes were recorded in our study (Electronic supplement S1). In general, the assemblages of genera were similar in field

and control microcosms at T0. There were nor significant differences in the richness of genera (Table 4, Fig. 6) neither in the number of individuals per genera, except in the case of rare genera (*Neochromadora*, *Oncholaimus*, *Oncholainellus*, *Halalaimus*, *Kosswigonema*, *Antomicron*, *Daptonema*, *Leptolaimus*, Morfotipo 1, *Theristus*) with higher abundance in Field with respect to T0 and *Sabatieria* with lower abundance in Field in compared to T0 (Table 4, Fig. 7). The dominant genus (*Pseudochromadora*) was the same in the field control and T0.

The number of genera (richness) was significantly different among treatments, with lower values in medium and high treatment respect to control. There were no significant changes over time in the genera richness in the controls, but in the treatments, both the medium and high treatment suffered a decrease in the number of genera over time (Table 4).

There was a significant multivariate effect of enrichment and time on the nematode assemblage (PERMANOVA significant Time x Treatments interaction: Pseudo- F= 2.735, Monte-Carlo p = 0.0006; 9920 permutations), showing that differences among treatments depend on the sampling time (Table 5 and Electronic supplement S2). After 15 days, significant differences were found only between medium and high enrichment treatments (t = 1.599, Monte-Carlo p = 0.05, 126 permutations); however, after 30 days, differences between control versus both medium and high enrichment treatments were significant (Monte-Carlo p = 0.0006, p = 0.006,126 permutations) (Table 5 and Electronic supplement S2).

The SIMPER procedure indicated that the same genera contributed to similarity within groups of controls at 4, 15 and 30 days: *Pseudochromadora* and *Terschellingia*. In addition, the average of dissimilarity between controls at different time did not exceed 20 %. The pair-wise comparisons between T0 and field showed also a significance difference, but the R value was low (R = 0.28, p = 0.04, 126 perm.), suggesting a small effect (Fig. 8, average of dissimilarity: 38%). The most marked differences (average of dissimilarity 50%) were observed in the pair-wise comparisons between enriched treatments at 4 days and 30 days.

There were significant effects of enrichment in the number of individuals of six out of ten genera (exception: *Oxystomina*, *Sabatieria*, *Paradontophora* and *Terschellingia*: Table 3). In the genera *Anonchus* and *Anoplostoma* there was a decrease in the number of individuals in both enriched treatments (high and medium) compared to the control

(Fig. 8). In *Viscosia* and *Paralinhomoeus* the number of individual decreased in the medium level of enrichment with respect to the control but increased under high level of enrichment compared to medium (Fig. 8). All genera significantly decreased in abundance along the experimental time, especially under high enrichment; by contrast, *Pseudochromador* increased its abundance with time, also especially under high enrichment conditions (Table 4, Fig. 8).

Community maturity and trophic diversity

The MI and ITD did not show significant differences among field and controls at day 0 (Table 4, Fig. 9). There was however, a significant temporal variation and a significant effect of organic enrichment on trophic structure of assemblages depending on time (significant interaction time x enrichment: Table 4), shown from ITD values driven by changes in deposit-feeders (groups 1A and 1B) as well as epigrowth feeders (2A). At the end of the experiment (T30) the trophic diversity decreased, which was driven by a decrease in the percentage associated to deposit-feeders (1B) and predator/omnivore (2B) accompanied by an increase in the proportion of epigrowth feeders (Fig. 9). In addition, a general decrease in trophic diversity and the increase of MI values along time in all treatments (significant time effect: Table 4, Fig. 9) were observed in both treatments. However, this increase in MI (to values 3 or 4), indicates lack of disturbance effects.

Discussion

In assessing the effect of enrichment on nematode assemblages we used a microcosom approach. We therefore increased our capacity to establish cause-effect relationships at the cost of losing realism. We minimized effects related to the construction of the laboratory assemblages, the so-called microcosm effect (leading to microhabitat homogenization temporal hypoxia and mortality of sensitive species): at T_0 assemblages did not differ from those in the field (Fig. 9) and both field conditions and the control at the beginning of the experiment had similarly aged and degraded sedimentary organic matter (PRT : CHO < 1: Dell'Anno et al. 2002), and low quality organic matter (CHO : LIP >>1). The fact that the enrichment effects were stronger than the effects of sediment disruption (see Table 1), validated our approach according to the criteria stated in Austen & McEvoy (1997). The environmental variables and the nematode assemblages varied little in the controls. The little variation in the nematode assemblage between field, T0

and controls was consistent with the fact that estuarine nematodes are robust to laboratory manipulation (Austen & McEvoy 1997) and elicit a minimal "microcosm effect" (e.g. Schratzberger et al. 2000, Hedfi et al. 2007). Overall, microcosm effects if exist would have led to immediate changes in the environment and biota. We conclude that the observed reductions in abundance, richness of genera and trophic richness and changes in trophic structure (loss of predators/omnivores and a dominance of selective deposit-feeders and epigrowth-feeders) in responses to enrichment as well as with reductions in oxygen concentration, are likely to occur under natural conditions given that we started our experiment with realistic nematode assemblages. We however recognize that further confirmation is needed through monitoring of natural assemblages and field experiments.

The experimental treatments were accurate in recreating organic enrichment and its consequences, in terms of trophic status of sediment and hypoxia. In our experiment we simulated an important input of labile organic matter with the addition of *Spirulina platensis*. Hence, as particles sank in the experimental units, there was an increase in Chl-a and proteins (detected as a significant interaction between enrichment by time. Table 2). There was also an increment in phaeopigments four days after the beginning of the experiment. There was also a change in the age of the organic matter, from aged to live/fresh, as quantified from the ratio of proteins to carbohydrates (PRT : CHO < 1 in controls and >1 in enriched treatments: Danovaro et al. 1993). Equally, the quality of organic matter increased as indicated from the increase in levels of BPC (Fabiano et al. 1995) and low CHO : LIP ratio (Joseph et al. 2008) driven by an increase in the concentration of lipids. The values of CHO : LIP (>>1) indicated that that the input of fresh organic matter had a low nutritional level albeit higher than the registered in controls. Enrichment also resulted in sediment hypoxia (oxygen concentrations < 2.8 mg l^{-1}: Diaz & Rosenberg 1995) in consistence with previous studies (Armenteros et al. 2010).The magnitude by hypoxia was higher over the first 15 day of the experiment (detected as significant interaction enrichment by time: Table 2). By contrast, most environmental variables varied little over time in the controls, with the exception of increases in organic matter and phaeopigments, but we expected such patterns, as over time, particles would sink slowly from the water column.

Enrichment resulted in a quick reduction of the total abundance (after 4 days), while the number of genera (i.e. richness) decreased only towards the end of the experiment (~30 days). This response was consistent with that found in other eutrophic estuaries (Netto & Valgas 2010, Armenteros et al. 2010). Nematode's response was not consistent with the

model proposed by Pearson & Rosenberg (1978). The model establishes that in high organic enrichment sediments the macrofauna is absent and nematodes are the dominant metazoans, predicting an initial increase in the species richness in response of enrichment followed by a subsequent increase in abundance as richness starts to decline. It may perhaps fits with the Dynamic Equilibrium Model whereby richness peaks at intermediate levels of disturbances and productivity (Huston 1979). According to this model, a decrease in species richness means that few opportunistic species become overabundant. Dominance may increase either as a consequence of competitive exclusion or as a consequence of fewer species tolerating the harsh conditions.

Most of the genera were affected by enrichment, but some responses were difficult to interpret and may reflect non-linear or complex responses to the multiple environmental changes associated to enrichment (e.g. changes in dissolved oxygen and organic matter composition). For instance, *Anoplostoma*, which decreased in abundance in both enrichment treatments, has been reported as favoured by organic enrichment (Kapusta et al. 2006). *Viscosia* composed mainly of facultative predators, able to exploit a wide range of food resources (Moens & Vincx 1997), had the lowest abundance at medium levels of enrichment and higher abundance at the high level of enrichment. The increase in density of *Pseudochromadora* in response to organic enrichment is more logical: these are epistrate feeders and benefit by the availability and diversity of food resources (Pinto & Bemvenuti 2003, Kapusta et al. 2006). Some genera (*Oxystomina*, *Sabatieria*, *Terschellingia* and *Paradontophora*) appeared to be tolerant to enrichment, as they did not show changes among treatments. This is consistent with previous studies showing that such genera are well known for their proliferation in stressful conditions or in close association with sediment organic enrichment (Mirto et al. 2002). Species of the genus *Terschellingia* are tolerant to a diversity of stressors in soft bottoms (Schratzberger et al. 2006); *Sabatieria* and *Oxystomina* are tolerant to aquaculture deposition; *Sabatieria* is well adapted to live in environments with high organic carbon loads, low oxygen, and high sulphide concentrations (Jensen et al. 1992, Soetaert & Heip 1995). *Parodontophora* species have been reported to be unresponsive to changes in chl-a sediment concentrations (Quang et al. 2016).

Enrichment also resulted in a reduction of the non-selective deposit-feeders (*Anoplostoma*, *Paralinhomoeus*) and predator/omnivores (*Viscosia*), which have faster metabolic rates, and presumably lower tolerance to hypoxia, than the epigrowth feeders (*Pseudochromadora*) and selective deposit-feeders (*Oxystomina*, *Terschellingia*) (Heip

et al. 1985), which would make them less tolerant to the hypoxia. The decline of predators could also be a consequence of the loss of habitat complexity, as the higher abundance of predators indicates a more heterogeneous and well-structured trophic assemblage that might imply a higher habitat complexity (Semprucci et al. 2015). Metabolic rates should be a key factor explaining the responses: selective deposit-feeders showed only a temporary decrease and epigrowth feeders increased under conditions of enrichment. The combination of low metabolic rates and the feeding mode may thus enable tolerance or proliferation under enrichment. Selective deposit-feeders take advantage of the food supply (Armenteros et al., 2010) until the most deleterious effects derived from the organic input occurs. Epigrowth feeders, common in estuaries (Ndaro & Ólafsson 1999) may be able to exploit a diversity of food sources available after enrichment; *Pseudochromadora*, the dominant genera in this trophic group consumes bacteria, microalgae and phytodetritus (Pinto & Bemvenuti 2003) and were clearly favored by proteins and high values of BPC.

We found clear responses of trophic groups due to organic enrichment, despite the controversy of assigning whole genera to different trophic groups (Heip et al. 1985). This classification strategy ignores the complexity of nematodes feeding habitats (Moens & Vincx 1997) and their trophic plasticity (Schratzberger et al. 2008). Most likely, the species composition and richness within each genera, and hence the likelihood of incorrectly assigning a specific organism to a particular trophic group, changes from site to site. It may well be that assemblages at Rocha Lagoon are dominated by a single species per genera which might drive the observed responses at the level of trophic groups. The effect of enrichment was also observed as a reduction in trophic richness (ITD index); which is contrary to with findings of other authors (Mirto et al. 2002, Alves et al. 2013) but consistent with Semprucci et al. (2013). Thus our study supports the hypothesis that enrichment alters nematode trophic structure. Nevertheless, we recognize the need of reevaluate the level of tolerance/sensitivity of the trophic groups to different stressors.

In contrast to the effects on trophic structure, enrichment did not seems to select for particular life history (as quantified from the index MI), perhaps as a result of a high percentage of k-strategists (c-p value of 3). MI was initially proposed for the study of terrestrial and freshwater habitats (Bongers 1990), and marine and brackish ecosystems were included later (Bongers et al. 1991), but the lack of empirical evidence regarding life strategies of most marine genera resulted in a conservative use of this index. MI is responsive to river discharge and is more efficient than diversity indices in detecting

effects of disturbance; however, it is also sensitive to sediment grain size (Semprucci et al. 2010, 2013). MI and c-p classes are sometimes unable to identify the dominant stressor when multiple stressors act together (Semprucci et al. 2013).

Given the multiple stressor nature of enrichment (organic matter content and quality is increased, but oxygen levels drop and driving the increases in concentrations of hydrogen sulfide and ammonia), we cannot identify which stressor drives the observed patterns in nematodes assemblages. Decreases in abundance may be driven mainly by hypoxia, as suggested by Gray et al. (2002) and Van Colen et al. (2009). Oxygen limitation is also suggested by the fact that the less responsive trophic groups were those characterized by low metabolic rates. Behavioral and physiological adaptations (e.g. migration to "oxygen islands": Balsamo et al. 2012; slow movement and low metabolic rates: Warwick & Price 1979, Warwick & Gee 1984) may explain why some groups did not were affected their abundances (or maintain their densities) In addition, tolerance hypoxia would have allow the access to organic matter in increased amount and quality (availability of food), which are key controlling factors of the growth, metabolism and distribution of benthic communities within the substrate (Danovaro & Fabiano 1997, Venturini et al. 2011).

In summary, our study showed that organic enrichment can drive changes in the trophic status of the sediments, reductions in abundance and richness of nematodes, the loss of predators/omnivores and the dominance of selective deposit-feeders and epigrowth feeders. Our results also suggest that the study of nematode assemblages at the genera level is enough to detect effects of enrichment, in consistence with other studies carried out elsewhere (Balsamo et al. 2012, Mirto et al. 2014), but also, that the index of trophic diversity seems to be a good candidate as an indicator of eutrophication effects on nematodes assemblages.

The extrapolation from the experiment to nature should be prudent since, this is one of the main sources of misleading conclusions (Carpenter 1996). However, the response of infauna to organic enrichment are governed primarily by the adaptations of species to conditions caused by organic load, thus extrapolation of responses from small-scale experiments to larger scale can be accepted (Zajac et al. 1998). In spite of that our experimental set-up probably amplified the effects of treatments because the stagnant conditions and the lack of water and sediment renewal, such amplification may be considered appropriate for a semi-enclosed coastal lagoon (Urban et al. 2009).

Acknowledgements

We thank our colleagues from Oceanografía y Ecología Marina, Facultad de Ciencias, UdelaR and CURE, Rocha, for their kind collaboration during sampling surveys and laboratory assistance. Special thanks go to Lic. Karen Iglesias for her help in byopolimeric analysis and MSc. Carolina Bueno for her help in one of the previous version of this manuscript. Kandratavicius was supported by Comisión Sectorial de Investigación Científica (CSIC) and P. Muniz and N. Venturini by SNI–ANII. Special thanks to the anonymous reviewers whose comments improved the quality of the manuscript.

References

Alves AS, Adão H, Ferrero TJ, Marques JC, Costa MJ & Patrício J (2013) Benthic meiofauna as indicator of ecological changes in estuarine ecosystems: The use of nematodes in ecological quality assessment. Ecol Indic 24: 462–475.

Armenteros M, Pérez-García JA, Ruiz-Abierno A, Díaz-Asencio L, Helguera Y, Vincx M & Decraemer W (2010) Effects of organic enrichment on nematode assemblages in a microcosm experiment. Mar Environ Res 70: 374-82.

Arocena R Fabián D & Clemente J (2000) Las causas naturales versus la contaminación orgánica como factores estructuradores del zoobentos en tres afluentes de una laguna costera. Limnética 18: 99-113.

Austen MC & McEvoy AJ (1997) The use of offshore meiobenthic communities in laboratory microcosm experiments: response to heavy metal contamination. J Exper Mar Biol Ecol 211: 247-261.

Balsamo M, Semprucci F, Frontalini F, Dr. Coccioni R (2012) Meiofauna as a tool for marine ecosystem biomonitoring marine ecosystems. In: Antonio C (ed) Marine ecosystems. In Tech, Rijeka, p 77–104.

Bongers T (1990) The Maturaty Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers T, Alkemade R & Yeates GW (1991). Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Mar Ecol Prog Ser 76: 135–142.

562 Byers S, Mills E & Stewart P (1978) Comparison of methods of determining
563 organic carbon in marine sediments, with suggestions for a standard method.
564 Hydrobiology 58: 43- 47.
565 Clarke KR & Gorley RN (2006) Change in marine communities: an approach to
566 statistical analysis and interpretation. 2nd ed. PRIMER-v6 Plymouth. 192p.
567 Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication
568 problem. Mar Ecol Prog Ser 210: 223–253.

569 Conde D & Rodríguez-Gallego L (2002) Problemática ambiental y gestión de las
570 lagunas costeras atlánticas de Uruguay. Perfil Ambiental 2002. In: Domínguez A & Prieto
571 R (eds.). NORDAN, Montevideo, p149-166.

572 Conde D, Aubriot L & Sommaruga R (2000) Changes in UV penetration
573 associated with marine intrusions and freshwater discharge in a shallow coastal lagoon of
574 the Southern Atlantic Ocean. Mar Ecol Prog Ser 207: 19–31.

575 Coull BC (1988) Ecology of the marine meiofauna. In: Higgins RP et al. (eds).
576 Introduction to the study of meiofauna. p 18-38.

577 Coull BC & Chandler GT (1992) Pollution and meiofauna: field, laboratory and
578 mesocosm studies. Oceanogr Mar Biol Ann\ Rev 30: 191-271.

579 Danovaro R (2010) Methods for the Study of Deep-Sea Sediments, Their
580 Functíoning and Biodíversity. CRC Press, New York, p 428.
581 Danovaro R & Fabiano M (1997) Seasonal changes in quality and quantity of food
582 available for benthic suspension-feeders in the Golfo Marconi (north-western
583 Mediterranean). Estuar Coast Shelf Sc 44: 723–736.
584 Danovaro R, Fabiano M & Della Croce N (1993). Labile organic matter and
585 microbial biomasses in deep-sea sediments (eastern Mediterranean Sea). Deep-Sea Res
586 40: 953–965.
587 Day JW, Hall CAS, Kemp WM & Yañez-Arancibia A (1989) Estuarine ecology.
588 Wiley & Sons Publications, New York, p558.
589 Dell'Anno A, Mei ML, Pusceddu A & Danovaro R (2002) Assessing the trophic
590 state and eutrophication of coastal marine systems: a new approach based on the
591 biochemical composition of sediment organic matter. Mar Pollut Bull 44: 611– 622

592 Diaz RJ & Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological
593 effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol Ann
594 Rev 33: 245–303.

595 Diaz RJ & Rosenberg R (2008) Spreading dead zones and consequences for
596 marine ecosystems. Science 321: 926-929.

597 Diaz RJ, Selman M & Chique C (2011) Global eutrophic and hypoxic coastal
598 systems, in: Eutrophication and Hypoxia: Nutrient Pollution in Coastal Waters,
599 Washington, DC, World Resources Institute. Available at:
600 http://www.wri.org/project/eutrophication.

601 Fabiano M & Danovaro R (1994) Composition of organic matter in sediments
602 facing a river estuary (Tyrrhenian Sea): relationships with bacteria and
603 microphytobenthic biomass. Hydrobiologia 277: 71-84.

604 Fabiano M, Danovaro R & Fraschetti S (1995) A three-year time series of
605 elemental and biochemical of organic matter in subtidal sand sediments of the Ligurian
606 Sea (Northwestern Mediterranean). Contin Shelf Res 15: 1453-1469.

607 Galois R, Blanchard G, Seguignes M, Huet V & Joassard L (2000) Spatial
608 distribution of sediment particulate organic matter on two estuarine mufllats: a
609 comparison between Marennes-Ole´ron Bay (France) and the Humber estuary. Contin
610 Shelf Res 20: 1199–1217.

611 Gerchacov SM & Hatcher PG (1972) Improved technique for analysis of
612 carbohydrates in the sediment. Limnol Oceanogr 17: 938-943.

613 Giménez L, Venturini N, Kandratavicius N, Hutton M, Lanfranconi A, Rodríguez
614 M, Brugnoli E & Muniz P (2014). Macrofaunal patterns and animal-sediment
615 relationships in Uruguayan estuaries and coastal lagoons (Atlantic coast of South
616 America). J Sea Res 87: 46–55.

617 Glasgow HB Jr & Burkholder JM (2000) Water quality trends and management
618 implications from a five-year study of a eutrophic estuary. Ecol Applic 10, 1024–1046.

619 Grall J & Chauvaud L (2002) Marine eutrophication and benthos: the need for
620 new approaches and concepts. Global Change Biol 8: 813-830.

621 Gray JS, Clarke KR, Warwick RM & Hobbs G (1990) Detection of initial effects
622 of pollution on marine benthos: an example from the Ekofisk and Edfisk oilfields, North
623 Sea. Mar Ecol Prog Ser 66: 285-299.

624 Gray JS, Wu RS, Or YY (2002) Effects of hypoxia and organic enrichment on the
625 coastal marine environment. Mar Ecol Prog Ser 238: 249-279.
626 Hargrave BT, Holmer M & Newcombe CP (2008) Towards a classification of
627 organic enrichment in marine sediments based on biogeochemical indicators. Mar Pollut
628 Bull 56: 810–824.
629 Hartree EF (1972) Determination of proteins: a modification of the Lowry method
630 that give a linear photometric response. Analytic Biochem 48: 422-427.
631 Hedfi A, Mahmoudi E, Boufahja F, Beyrem H & Aïssa P (2007) Effects of
632 increasing levels of nickel contamination on structure of offshore nematode communities
633 in experimental microcosms. Bull Environ Contam Toxicol 79: 345-349.
634 Heip PC, Vincx M & Vranken G (1985) The ecology of marine nematodes.
635 Oceanogr Mar Biol Ann Rev 23: 399-489.
636 Herreid CF (1980) Hypoxia in invertebrates. Comp Biochem Physiol 67:
637 311-20.
638 Huston MA (1979) A general hypothesis of species diversity. Amer Naturalist
639 113: 81–101.
640 Jensen P, Rumohr J & Graf G (1992) Sedimentological and biological differences
641 across a deep-sea ridge exposed toadvection and accumulation of fine-grained particles.
642 Oceanol Acta 15:287–296.
643 Joseph MM, Ratheesh Kumar CS, Greesh Kumar TR, Renjith KR &
644 Chandramohanakumar N (2008) Biogeochemistry of surficial sediments in the intertidal
645 systems of a tropical environment. Chem Ecol 24: 247-258.
646 Kandratavicius N, Muniz P, Venturini N & Giménez L (2015) Meiobenthic
647 communities in permanently open estuaries and open/closed coastal lagoons of Uruguay
648 (Atlantic coast of South America). Estuar Coast Shelf Sc 163: 44–53.
649 Kapusta SC, Wurdig NL, Bemvenuti CE & PintoTK (2006) Spatial and temporal
650 distribution of nematoda in a subtropical estuary. Acta Limnologica Brasiliensis
651 182:133–144.
652 Kelly JR & Nixon SW (1984) Experimental studies of the effect of organic
653 deposition on the metabolism of a coastal marine bottom community. Mar Ecol Prog Ser
654 17:157-169.

655 Kendall MA, Davey JT & Widdicombe S (1995) The response of two estuarine
656 benthic communities to the quantity and quality of food. Hydrobiologia 311: 207-214.

657 Keeling R F, Kortzinger A, & Gruber N (2010) Ocean deoxygenation in a
658 warming world. Ann Rev Mar Sc 2: 199–229.

659 Kjerfve B & Magill K (1989) Geographic and hydrodynamic characteristics of
660 shallow coastal lagoons. Mar Geol 88: 187–199.

661 Lorenzen CJ (1967) Determination of chlorophyll and pheopigments:
662 Spectrofotometric equations. Limnol Oceanogr. 12: 343-346.

663 Marsh JB & Weinstein DB (1966) Simple charring methods for determination of
664 lipids. J Lip Res 7: 574-576.

665 Mirto S, La Rosa T, Gambi, C, Danovaro R & Mazzola A (2002) Nematode
666 community response to fih-farm impact in the western Mediterranean. Environ Pollut
667 116: 203-214.

668 Mirto S, Arigò C, Genovese L, Pusceddu A, Gambi C & Danovaro, R (2014)
669 Nematode assemblage response to fish-farm impact in vegetated (Posidonia oceanica)
670 and non-vegetated habitats. Aquacul Envir Interactions, 5: 17-28.

671 Moens T & Vincx M (1997) Observations on the feeding ecology of estuarine
672 nematodes. J Mar Biologic Ass UK 77: 211–227.

673 Ndaro GM & Olatsson E (1999) Soft-bottom fauna with emphasis on nematode
674 assemblage structure in a tropical intertidal lagoon in Zanzibar, eastern Africa: I. spatial
675 variability. Hydrobiologia, 405: 133-148.

676 Netto SA & Valgas I (2010) The response of nematode assemblages to intensive
677 mussel farming in coastal sediments (Southern Brazil). Environ Monit Assess 162:81–93

678 Nilsson P, Jonsson B, Swanberg IL & Sundback K (1991) Response of marine
679 shallow-water sediment system to an increased load of inorganic nutrients. Mar Ecol Prog
680 Ser 71: 275–290.

681 Norling K, Rosenberg R, Hulth S, Grémare A & Bonsdorff E (2007) Importance
682 of functional biodiversity and species-specific traits of benthic fauna for ecosystem
683 functions in marine sediment. Mar Ecol Prog Ser 332: 11-23.

684 Pearson TH & Rosenberg R (1978) Macrobenthic succession in relation to organic
685 enrichment and pollution of the marine environment. Oceanogr Mar Biol Ann Rev 16:
686 229-311.

687 Perissinotto R, Stretch DD, Whitfield AK, Adams JB, Forbes AT & Demetriades
688 NT (2010) Ecosystem functioning of temporarily open/closed estuaries in South Africa.
689 In Crane JR &Solomon A E (eds) Estuaries: Types, Movement Patterns and Climatical
690 Impacts. Nova Science Pub Inc. Chapter 1, p 70.

691 Pinto TK & Bemvenuti CE (2003) Changes in Nematoda Community Structure
692 Related to Sewage discharge. Journal of Coastal Research, SI 39 (Proccendigs of the 8th
693 International Coastal Symposium), Itajaí, SC – Brazil, ISSN 0749-0208.

694 Pita AL, Gimenez L, Kandratavicius N, Muniz P & Venturini N (2017). Benthic
695 trophic status of aquatic transitional environments with distinct morphological and
696 dynamic characteristics on the South-western Atlantic coast. Mar Freshwat Res DOI:
697 10.1071/MF16284.

698 Platt HM & Warwick RM (1983) Free living marine nematodes. Parte I British
699 Enoplids. Published for The Linnean Society of London & The Estuarine & Brackish-
700 Water Sciences Association. Cambridge University Press.

701 Platt HM & Warwick RM (1988) Free living marine nematodes. Parte II British
702 Chromadorids. Synopses of the British Fauna (New Series). Kermack DM & Barnes RSK
703 (Eds.). Published for The Linnean Society of London & The Estuarine & Brackish-Water
704 Sciences Association.

705 Pusceddu A, Dell'Anno A, Fabiano M & Danovaro R (2009) Quantity and
706 bioavailability of sediment organic matter as signatures of benthic trophic status. Mar
707 Ecol Prog Ser 375: 41-52.

708 Quang N X, ChauN N, Smol N & Prozorova L & Vanreusel A (2016) Intertidal
709 nematode communities in the Mekong estuaries of Vietnam and their potential for
710 biomonitoring. Environ Monit Assess 188:91.

711 Rice DJ (1982) The detritus nitrogen problem: new observations and perspectives
712 from organic geochemistry. Mar Ecol Prog Ser 9:153-162.

713 Ríos A F, Fraga F, Pérez F.F &. Figueiras F G (1998) Chemical composition of
714 phytoplankton and Particulate Organic Matter in the Ría de Vigo (NW Spain) Sci.
715 Mar.62: 257-271

716 Rodríguez-Gallego L, Santos C, Amado S, Gorfinkel D, González MN, Gómez J,
717 Neme C, Tommasino H & Conde D (2008) Costos y beneficios socioeconomicos y
718 ambientales del uso actual de la Laguna de Rocha y su cuenca: insumos para la gestión
719 integrada de un área protegida costera. PROYECTO 36-09. Programa de desarrollo
720 tecnológico. Informe Final.

721 Ruhl HA, Ellena JA, Smith KL Jr (2008) Connections between climate, food
722 limitation, and carbon cycling in abyssal sediment communities. Proc Nat Acad Sc USA.
723 105:17006–17011.

724 Sampou P & Oviatt CA (1991) Seasonal patterns of sedimentary carbon and
725 anaerobic respiration along a simulated eutrophication gradient. Mar Ecol Prog Ser
726 72:271-282.

727 Schratzberger M & Warwick RM (1998) Effects of physical disturbance on
728 nematode communities in sand and mud: a microcosm experiment. Mar Biol 130: 643-
729 650.

730 Schratzberger M, Rees HL & Boyd SE (2000) Effects of simulated deposition of
731 dredged material on structure of nematode assemblages: the role of burial. Mar Biol 136:
732 519-530.

733 Schratzberger M, Warr K, Rogers SI (2006) Patterns of nematode populations in
734 the southwestern North Sea and their link to other components of the benthic fauna. J Sea
735 Res 55: 113-127.

736 Schratzberger M, Forster RM, Goodsir F& Jennings S (2008) Nematode
737 community dynamics over an annual production cycle in the central North Sea. Mar
738 Environ Res 66: 508-519.

739 Semprucci F, Colantoni P, Baldelli G, Rocchi M & Balsamo M (2010) The
740 distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean).
741 Mar Ecol 31: 592–607.

742 Semprucci F, Moreno M, Sbrocca S, Rocchi M, Albertelli G & Balsamo M. (2013)
743 The nematode assemblage as a tool for the assessment of marine ecological quality status:
744 a case-study in the Central Adriatic Sea. Mediterranean Mar Sc 1: 48–57.

745 Semprucci F, Losi V & Moreno M (2015) A review of Italian researchon free-
746 living marine nematodes and the future perspectives on their use as ecological indicators
747 (EcoInds). Mediterranean Mar Sc 16: 352–365.

748 Soetaert K & Heip CHR (1995) Nematode assemblages of deepsea and shelf break
749 sites in the North Atlantic and Mediterranean Sea. Mar Ecol Prog Ser 125:171–138.

750 Somerfield P & Warwick R (1996). Meiofauna in Marine Pollution Monitoring
751 Programmes: a Laboratory Manual. MAFF Directorate of Fisheries Research Technical
752 Series.

753 Snelgrove PVR & Butman CA (1994) Animal-sediment relationships revisited:
754 cause versus effects. Oceanogr Mar Biol An Ann Rev 32:111-177.

755 Sündback K (1983) Microphytobenthos on sand in shallow brackish water.
756 Öresund Sweeden. Primary production, chlorophyll a content and spices composition
757 (Diatom) in relation to some ecological factors. Tesis Doctoral. Department of Systematic
758 Botany, University of Lund, Sweeden. LUNDS/ (NBBS-1006): p 209.

759 Sundbäck K, Jonsson B, Nilsson P & Lindström I (1990) Impact of accumulating
760 drifting macroalgae on a shallow-water sediment system: an experimental study. Mar
761 Ecol Prog Ser 58: 261-274.

762 Urban ER, Sundbay B, Malanotte-Rizzoli P & Melillo JM (2009) Watersheds,
763 Bays and Bounded Seas. Island Press, Washington.

764 Van Colen C, Montserrat F, Verbist K, Vincx M, Steyaert M, Vanaverbeke J,
765 Herman PMJ, Degraer S & Ysebaert T (2009) Tidal flat nematode response to hypoxia
766 and subsequent macrofauna-mediated altered sediment properties. Mar Ecol Prog Ser
767 381: 189–197.

768 Vemberg, FJ (1972) Dissolved gases. Animals. In: Kinne O (ed.) Marine ecology,
769 Vol. 1, Pan 3, p 1491-1526. London: Wiley-Interscience.

770 Venturini N, Pires-Vanin AMS, Salhi M, Bessonart M & Muniz P (2011).
771 Polychaete response to fresh food supply at organically enriched coastal sites:
772 repercussion on bioturbation potential and trophic structure. J Mar Systems 88: 526–541.

773 Venturini N, Pita AL, Brugnoli E, Garcia-Rodriguez F, Burone L, Kandratavicius
774 N, Hutton M & Muniz P (2012) Benthic trophic status of sediments in a metropolitan area
775 (Rio de la Plata estuary): linkages with natural and human pressures. Estuar Coast Shelf
776 Sc 112: 139 – 152.

777 Vincx M (1996) Meiofauna in marine and freshwater sediments. In: Hall GS (Ed.)
778 Methods for the Examination of Organismal Diversity in Soils and Sediments. Cab
779 International, Wallinfort, UK, p 187–195.

780 Warwick RM & Price R (1979) Ecological and metabolic studies on free-living
781 marine nematodes from an estuarine mud-flat. Estuar, Coast Shelf Sc 9: 257-271.

782 Warwick, R.M., Gee, J.M., 1984. Community structure of estuarine meiobenthos.
783 Mar Ecol Prog Ser 18: 97-111.

784 Warwick RM, Carr MR, Clarke KR, Gee JM & Green RH (1988) A mesocosm
785 experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-
786 sediment meiobenthic community. Mar Ecol Prog Ser. 46: 181–191.

787 Warwick RM, Platt HM & Sommerfield PJ (1998) Free-living nematodes (Part
788 III) Monhysterids. In: Synopsis of British Fauna, No. 53. Barnes and Crothers.

789 Weston DP (1990) Quantitative examination of microbenthic community changes
790 along an organic enrichment gradient. Mar Ecol Prog Ser 61: 233-244.

791 Wieser W (1953) Die Beziehung zwischen Mundhöhlengestalt, Ernährungswiese
792 und 1000 Vorkommen bei freilebenden marinen Nematoden. Arkiv för zoology. 4: 439–
793 484.

794

795 **TABLES**

796 **Table 1.** Results of least square means (t-test) (1) field *vs.* T_0 to test the differences
797 between field and the experimental conditions; (2) T_0 *vs.* controls at time 4, 15 and 30
798 (C4, C15, C30) to test the temporal changes in the microcosm controls. This test were
799 apply in the variables with significant one-way ANOVA.

	Field vs T0	**T0 vs C4**	**T0 vs C15**	**T0 vs C30**
OM	t4=1.37, 0.242	t4=-1.11, 0.327	t4=6.45, 0.003*	t4=-2.81, 0.04*
Chl-a	t4= 3.35, 0.028*	t4=-4.22, 0.01*	t4=8.32,0.001*	t4=-1.04, 0.35
Pheopig	t4=4.63, 0.009*	t4=0.31, 0.77	t4=6.17, 0.003*	t4=-11.7, 0.0002*
CHO	t4=4.17, 0.014*	t4=-1.52, 0.20	t4=2.91, 0.04	t4=-1.07, 0.34

800

801 **Table 2.** Size effects expressed as % Increase (T0 vs Field and T0 vs Enrichment) in
802 chlorophyll a (Chl-a), phaeopigments, organic matter (OM) and biopolymers (CHO, PRT,
803 LIP and BPC). Where M= Medium, H= High and 4, 15 and 30 represents time.

Increase (%)	**Chl-a**	**Phaeopig**	**OM**	**CHO**	**PRT**	**LIP**	**BPC**
T0-Field	78	100	32	25	9	-1	12
T0-M4	524	1197	109	13	19	52	39

T0-M15	953	1867	76	7	49	0.4	37
T0-M30	1367	2877	132	1	56	27	45
T0-H4	1032	2911	181	0.3	32	30	34
T0-H15	964	3044	200	25	58	49	61
T0-H30	2214	6303	303	25	61	42	61

804

805 **Table 3.** Results of statistical comparisons of univariate measures of abiotic components.
806 Values of statistic F with degrees of freedom and p-values. For all variables except
807 dissolved oxygen the data was analysed through a standard two-way crossed ANOVA,
808 considering treatment and sampling time as factors. Oxygen data were analysed using
809 within subject (repeated measures) ANOVA considering Time as a within subject factor
810 and treatment as a between subject factor; in this case, there are three separate analyses
811 by time comparing daily measures of oxygen level. * significant difference.

Factor	Treatment ($F_{2,18}$)	Time ($F_{2,18}$)	Treatment x Time ($F_{4,18}$)
OM	12.37, < 0.0001*	2.78, 0.089	1.48, 0.25
Chlorophyll a	85.03, < 0.0001*	7.47, 0.04*	4.1, < 0.016*
Phaeopigments	344.7, < 0.00001*	21.3, < 0.0001*	1.7, 0.202
BPC	30.01, < 0.0001*	3.4, 0.056	2.12, 0. 121
Proteins	58.69, < 0.00001*	13.96, 0.00*	3.86, 0.02*
Lipids	10.68, < 0.0001*	0.16, 0.855	1.82, 0.17
Carbohydrates	1.007, 0.385	0.354, 0.707	1.41, 0.271
O_2 Time 4	$F_{(2, 12)} = 529.34$, $p < 0.00001$*	$F_{(4, 48)} = 24.545$, $p < 0.00001$*	$F_{(8, 48)} = 19.607$, $p < 0.00001$*
Time 15	$F_{(2, 12)} = 171.82$, $p < 0.00001$*	$F_{(14, 168)} = 11.779$, $p < 0.00001$*	$F_{(28,168)} = 8.921$, $p < 0.00001$*
Time 30	$F_{(2, 12)} = 53.542$, $p < 0.00001$*	$F_{(27, 324)} = 46.642$, $p < 0.00001$*	$F_{(54,324)} = 12.788$, $p < 0.00001$*

812

813 **Table 4.** Results of statistical comparisons of univariate measures of nematode
814 assemblages. Values of statistic F and probability of the two type of ANOVAs: one-way
815 (Field *vs.* T0) and two-way crossed, are shown. Feeding Type: 1A= selective deposit-
816 feeder, 1B= non-selective deposit-feeder, 2A= epigrowth feeder and 2B=
817 omnivore/predator. * significant difference.

Factor	Treatment ($F_{2,36}$)	Time ($F_{2,36}$)	Treatment x Time ($F_{4,36}$)	Field *vs* To ($F_{1,8}$)
Nematodes	17.11, 0.00*	4.8, 0.014*	3.95, 0.009*	1.25, 0.297
Anonchus	13.38, 0.00*	10.44, 0.00*	1.08, 0.381	2.89, 0.128
Anoplostoma	5.01, 0.012*	15.4, 0.00*	4.32, 0.006*	5, 0.056
Oxystomina	2.985, 0.063	0.002, 0.998	1.791, 0.152	2.51, 0.152
Pseudochromadora	12.04, 0.00*	27.27, 0.00*	5.86, 0.001*	0.256, 0.626
Paradontophora	2.537, 0.093	6.805, 0.003*	2.154, 0.094	4.5, 0.067
Paralinhomoeus	4.544, 0.017*	1.931, 0.16	0.504, 0.733	1.76, 0.221

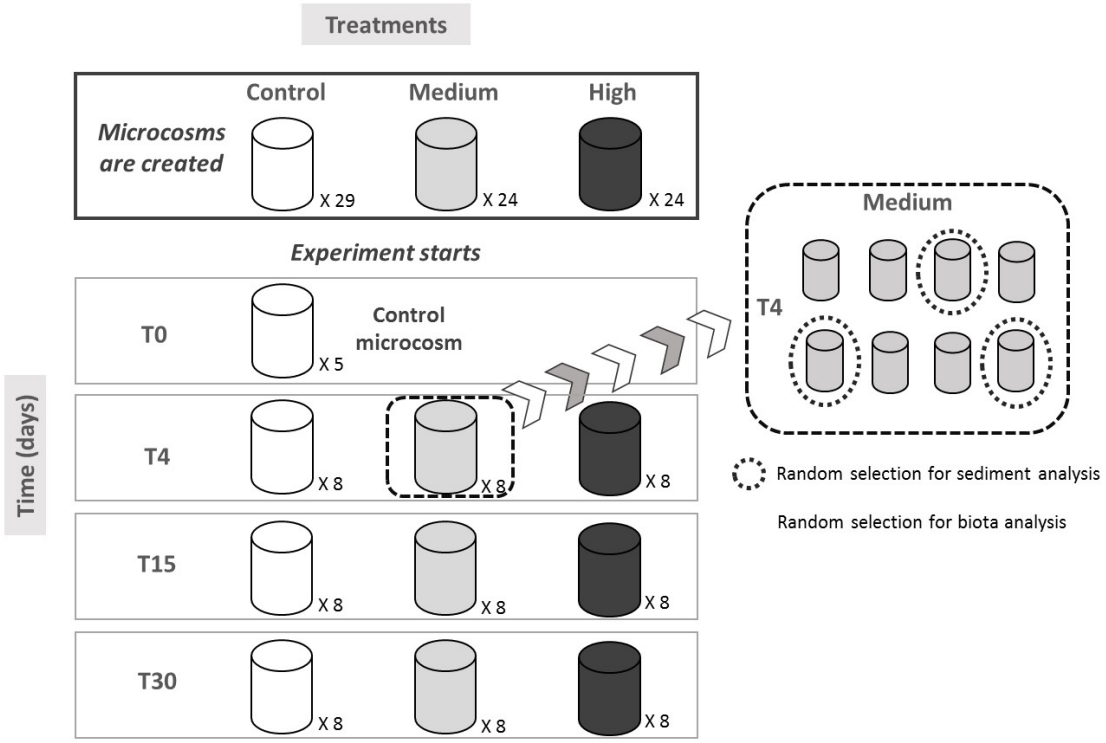
Sabatieria	1.95, 0.156	11.51, 0.00*	5.6, 0.001*	9.09, 0.017*
Terschellingia	0.964, 0.391	1.581, 0.222	2.568, 0.054	0.61, 0.459
Viscosia	7.14, 0.002*	26.87, 0.00*	1.03, 0.404	0.276, 0.614
Rare genera	3915, 0.029*	5.06, 0.012*	1.70, 0.17	7.86, 0.023*
Richness of genera	9.25, 0.00*	17.79, 0.00*	3.37, 0.019*	3.368, 0.104
ITD	12.56, 0.00*	32.64, 0.00*	7.52, 0.00*	0.255, 0.627
1A	2.269, 0.118	2.295, 0.115	4.02, 0.009*	0.116, 0.745
1B	3.39, 0.045*	15.53, 0.00*	3.84, 0.011*	0.857, 0.39
2A	6.68, 0.003*	18.78, 0.00*	4.48, 0.005*	0.437, 0.533
2B	7.04, 0.003*	27.88, 0.00*	1.21, 0.325	0.023, 0.884
MI	0.353, 0.705	7.412, 0.002*	1.941, 0.125	0.191, 0.63

Table 5. p-values based on PERMANOVA testing for responses of nematodes to enrichment (control, medium and high) and time. Significant values ($p < 0.05$) are highlighted with *.

	df	SS	MS	Pseudo-F	p (Montecarlo)	perm.	p(perm)
Treatment	2	1799	899.5	5.5485	0.0001*	9936	0.0001*
Time	2	3394.5	1697.2	10.469	0.0001*	9930	0.0001*
Treatment x Time	4	1773.8	443.45	2.7354	0.0006*	9920	0.0003*
Residual	36	5836.2	162.12				
Total	44	12803					

	df	SS	MS	Pseudo-F	p (Montecarlo)	permutations
Treatment	2	1799	899.5	5.5485	0.0001*	9936
Time	2	3394.5	1697.2	10.469	0.0001*	9930
Treatment x Time	4	1773.8	443.45	2.7354	0.0006*	9920
Residual	36	5836.2	162.12			
Total	44	12803				

FIGURES



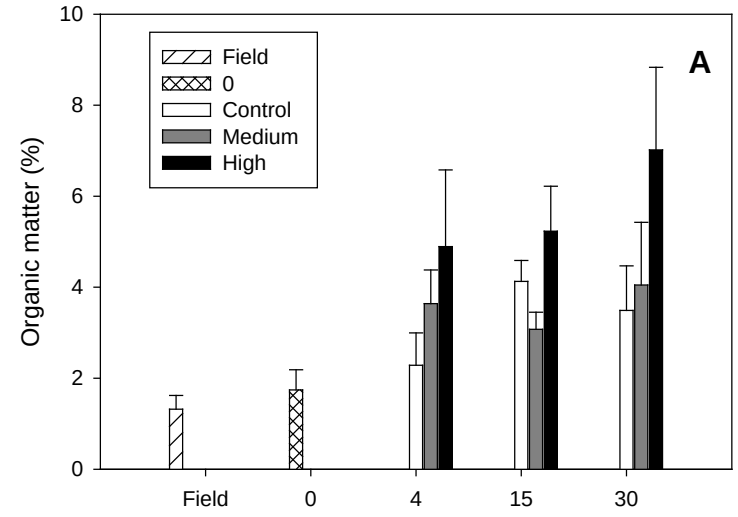
824

825

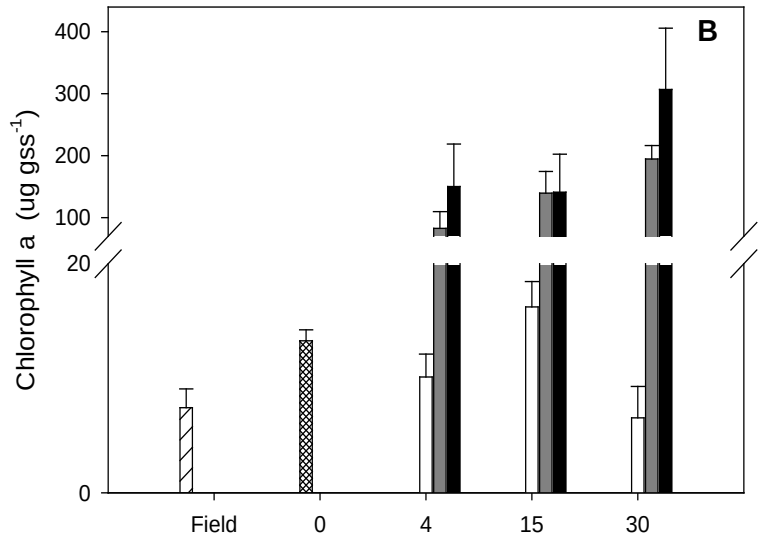
826 **Fig. 1.**

827

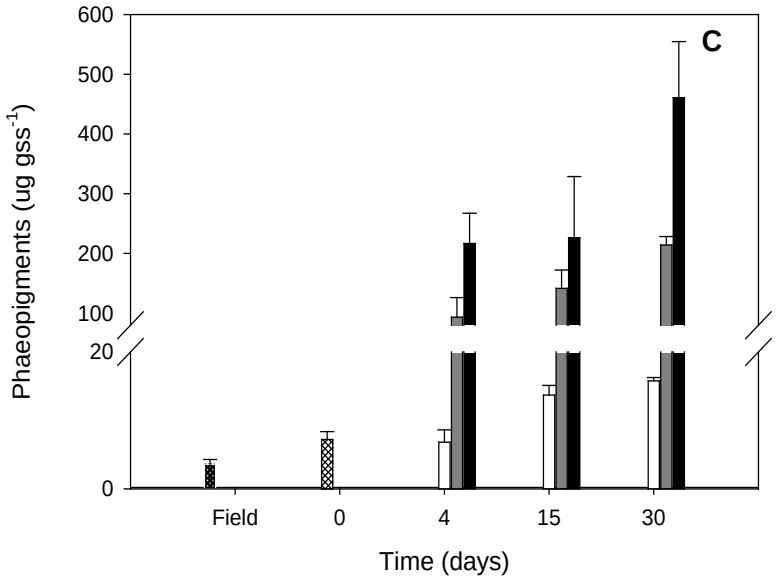
828



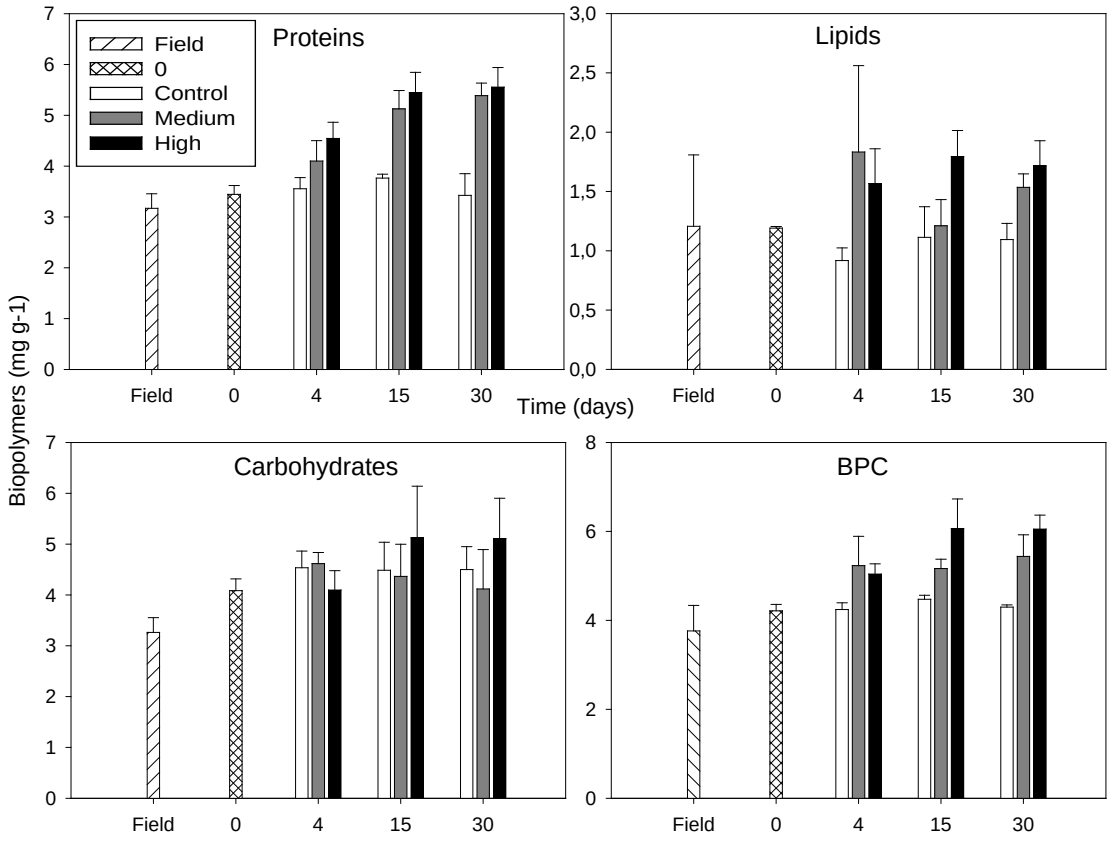
829



830

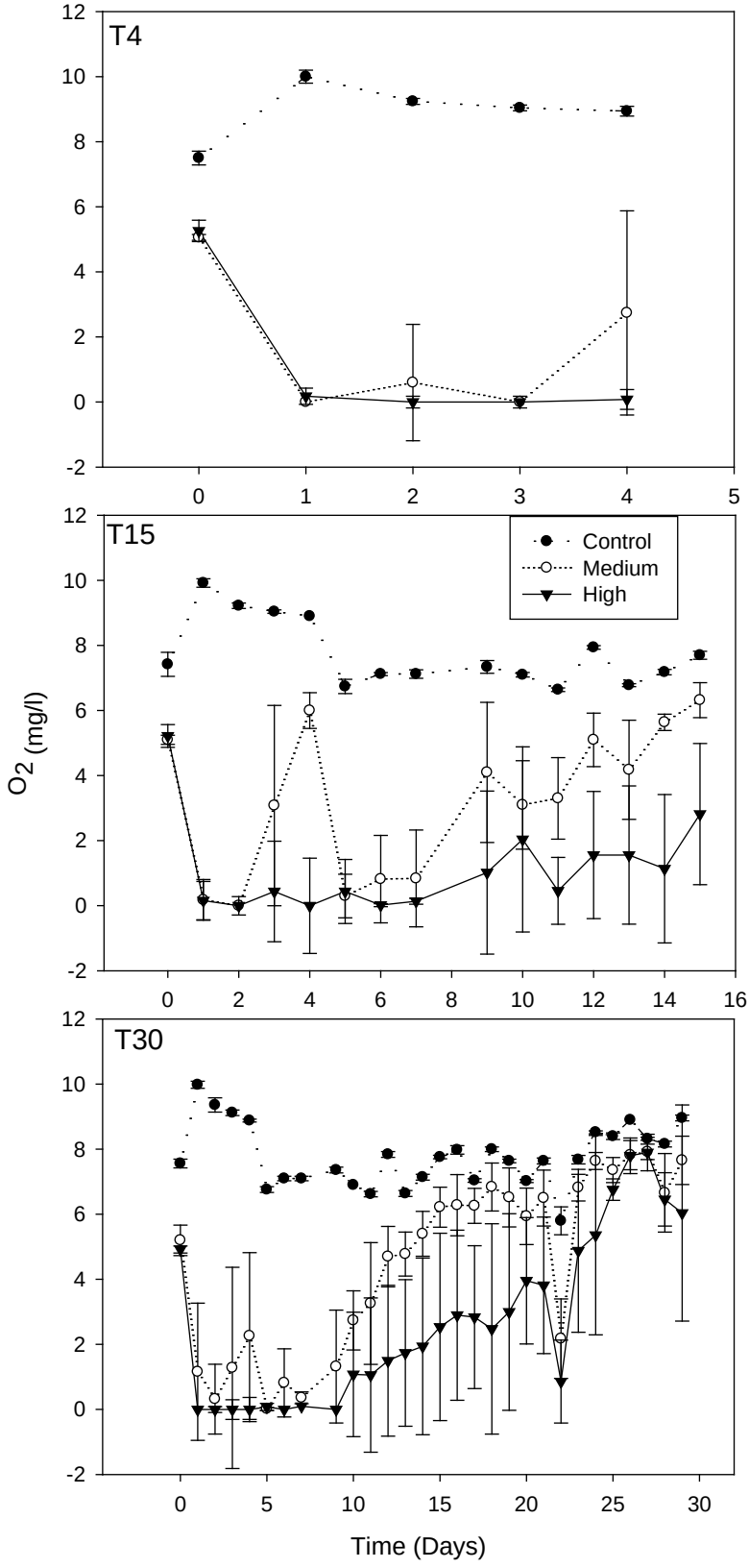


831 **Fig. 2.**


Field
0
Control
Medium
High
Proteins
Lipids
Carbohydrates
BPC
Biopolymers (mg g-1)
Time (days)
Field
0
4
15
30

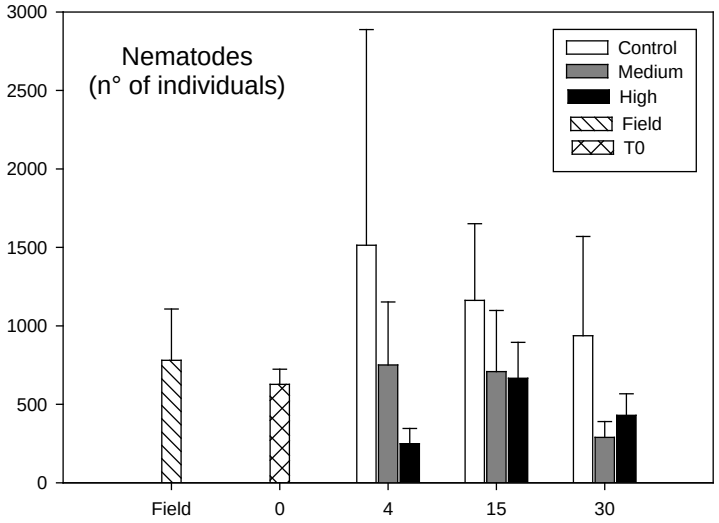
832

833 **Fig. 3.**



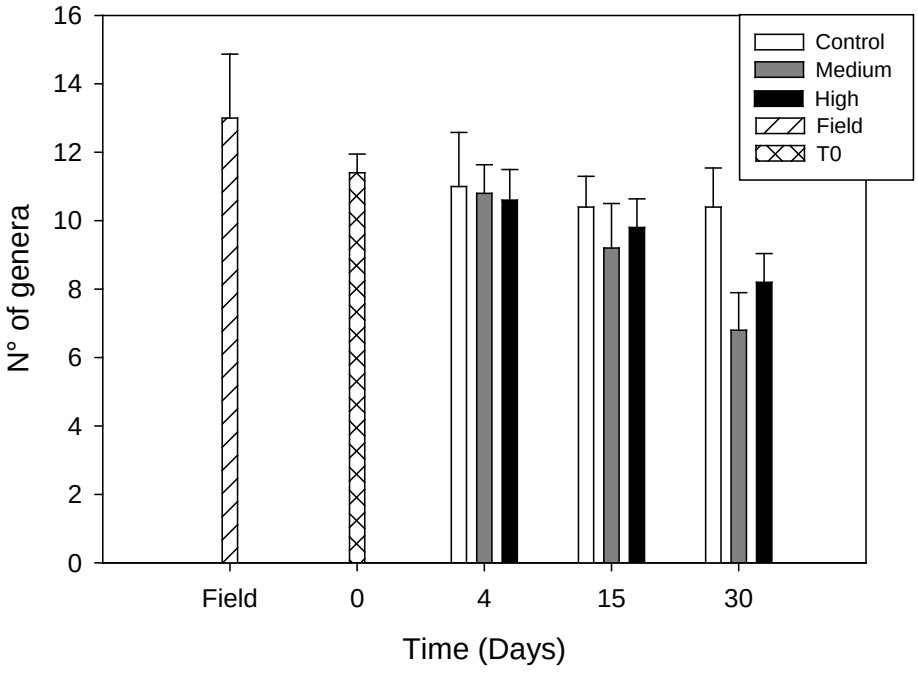
834

835 **Fig. 4.**



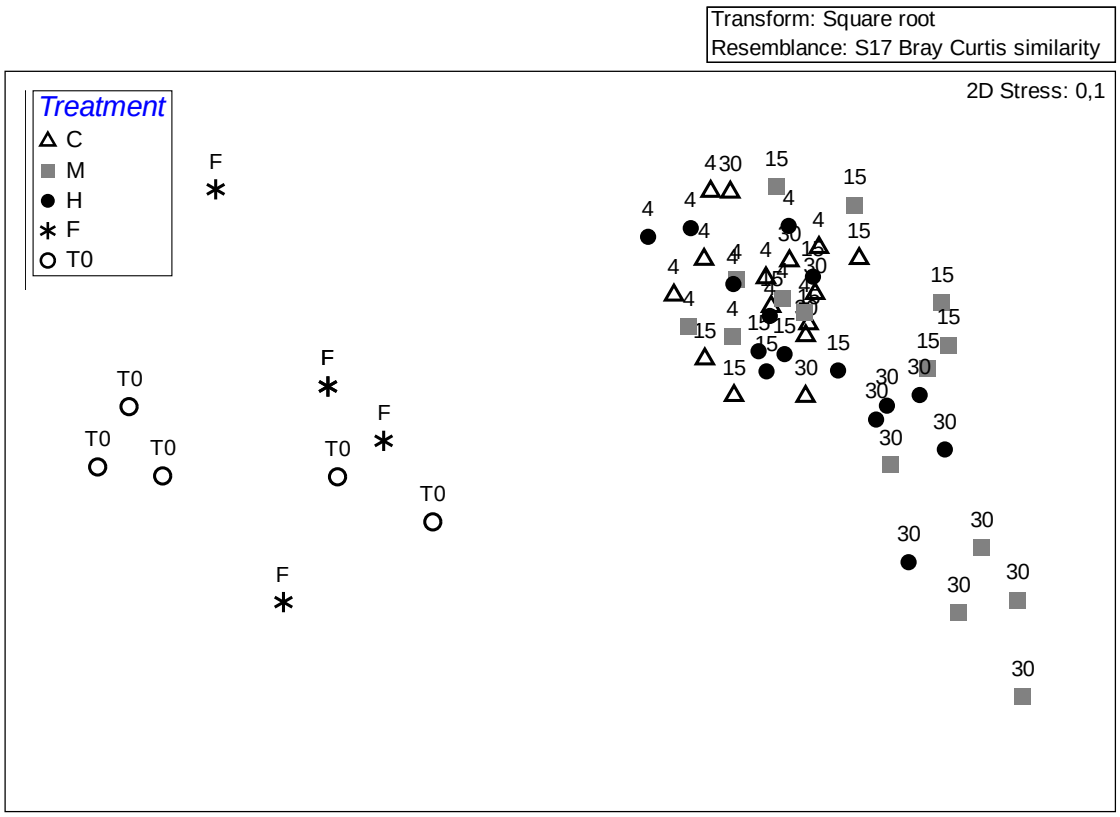
836

837 **Fig. 5.**



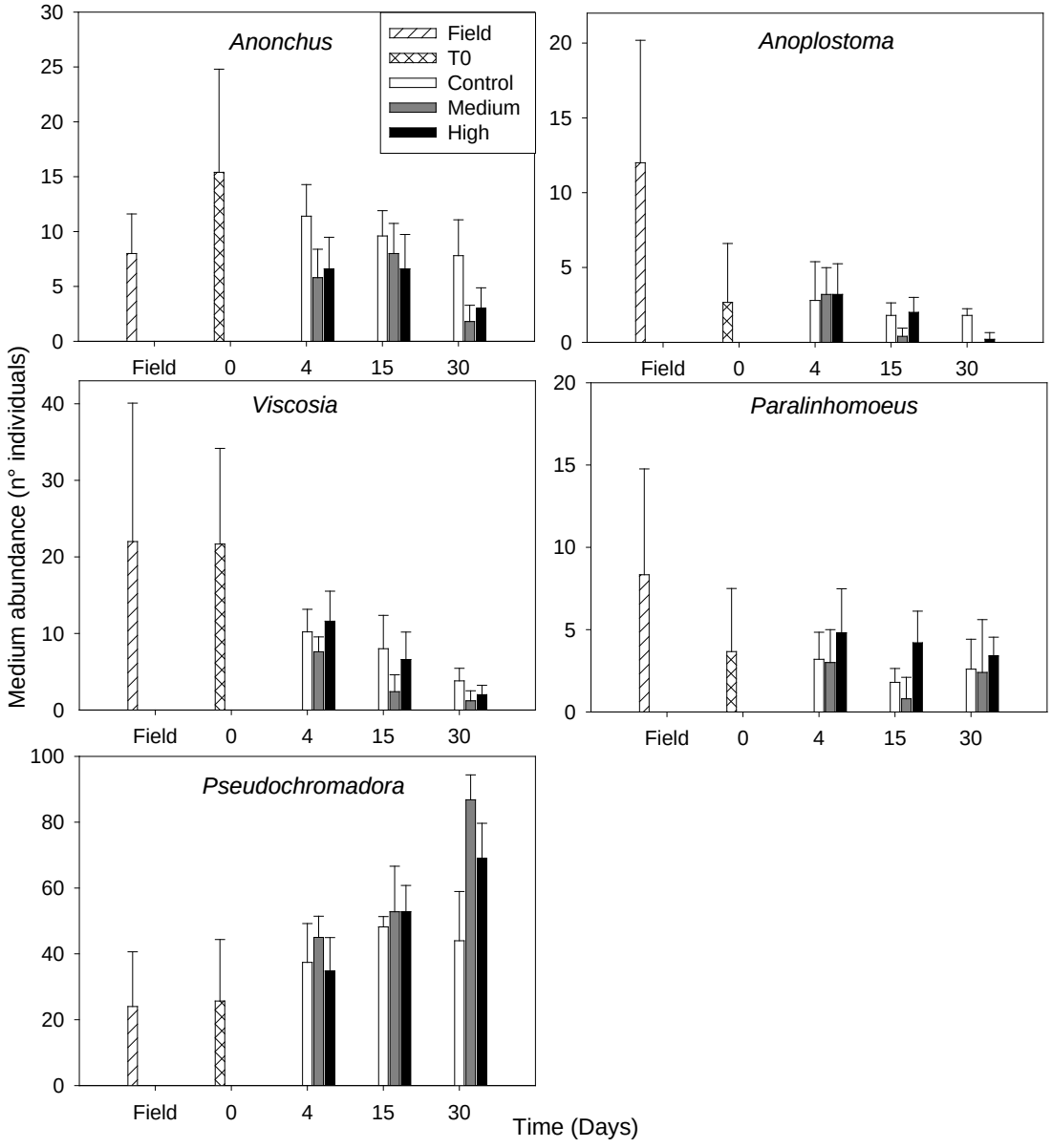
838

839 **Fig. 6.**


Transform: Square root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0,1
Treatment
C
M
H
F
T0

840

841 **Fig. 7.**


Anonchus
Field
T0
Control
Medium
High
Anoplostoma
Viscosia
Paralinhomoeus
Pseudochromadora
Medium abundance (n° individuals)
Time (Days)
Field
0
4
15
30

842

843 **Fig. 8.**

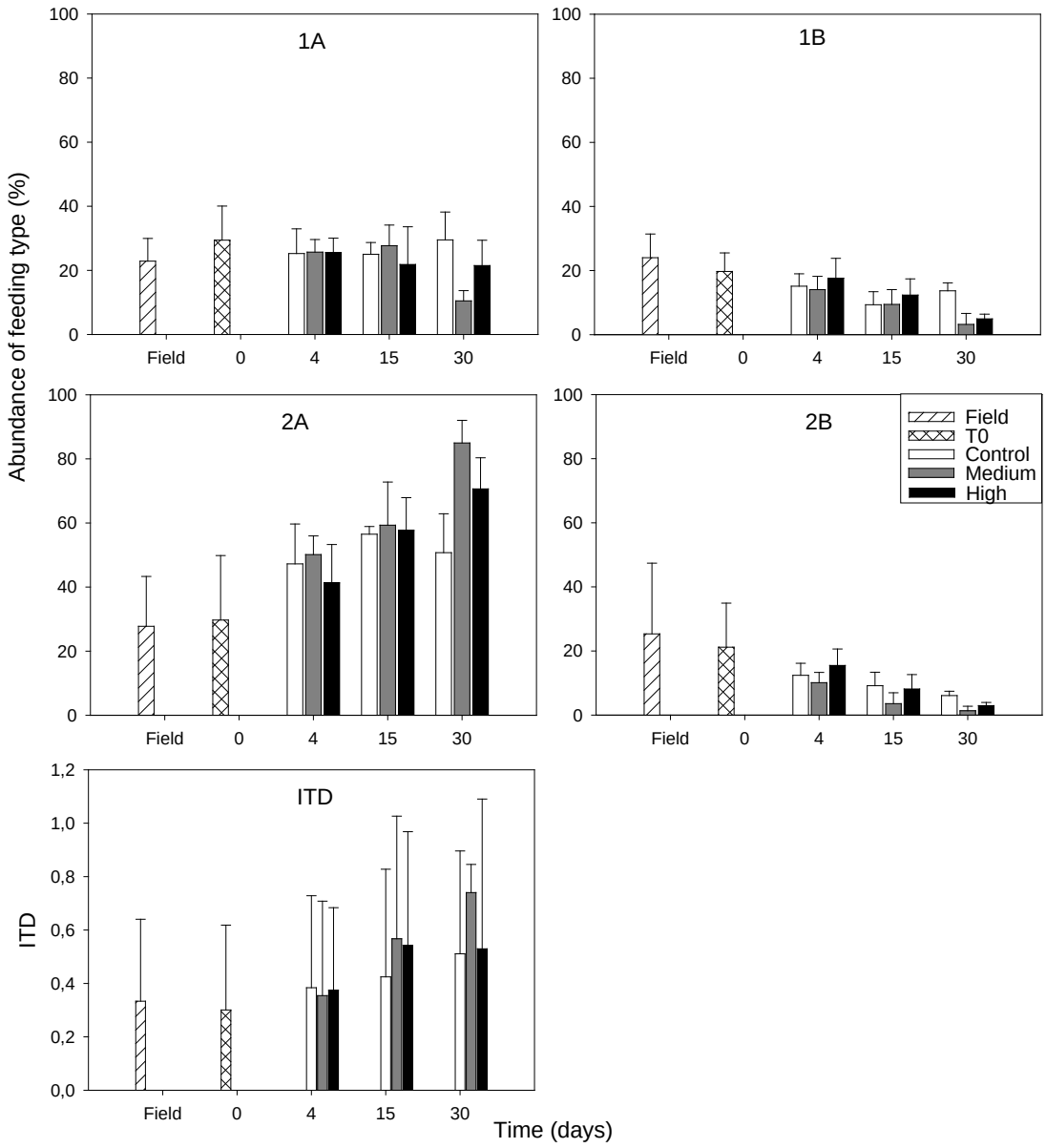


Fig. 9.

CAPTIONS

Fig. 1. Experimental design. Three different treatments were applied: Medium (2.5g *S. platensis*), High (5g *S. platensis*) and Control (no *S. platensis).* At day 0 five microcosms of control treatment were taken to analyze the initial structure of nematode assemblage (Microcosms control). At days 4, 15 and 30, 24 microcosms (8 replicates per treatment) were random extracted. From 8 replicated microcosms per treatment, five were taken for the analysis of nematodes structure and two for the chemical analysis of sediment.

Fig. 2. Mean values and SD of abiotic factors measured from a field site, Time 0 and microcosms treatments (control, medium and high) at different time (4, 15 and 30 days). A) Total organic matter. B) Chlorophyll a. C) Phaeopigments.

Fig. 3. Mean values and SD of biopolymers (PRT: proteins, CHO: carbohydrates, LIP: lipids and BPC: Biopolymeric carbon) measured from a field site, Time 0 and microcosms treatments (control, medium and high) at different time (4, 15 and 30 days).

Fig. 4. Mean values and SD of dissolved oxygen from microcosms treatments at time. Where T4: microcosm withdrawn four days, T15: microcosm withdrawn 15 days and T30: microcosm withdrawn 30 days.

Fig. 5. Mean values and SD of abundance of nematodes measured from field site, Time 0 and microcosms treatments (control, medium and high) at different time (4, 15 and 30 days).

Fig. 6. Richness of genera (mean and SD) of nematode assemblages in sediments from Field site, control at day 0, control and treatments (medium and high) at time (days 4, 15 and 30).

Fig. 7. Non metric multidimensional scaling ordination of samples based on square-root transformed data of density of nematode genera in sediment from: field (F), time 0 (T0), control (C), medium treatment (M) and high treatment (H). Number upper symbol indicates days after the onset of the experiment.

Fig. 8. Average abundance of the main genera in sediments from a field site, control at T0, control and treatments (medium and high) at different time (4, 15, 30 days).

Fig. 9. Average percentage of ITD value and feeding types of nematode assemblages in sediments from field site, control at T0, control and treatments at different time (4, 15 and 30 days). Feeding types after Wieser (1953): 1A= selective deposit-feeder, 1B= non-selective deposit-feeder, 2A= epigrowth-feeder, 2B= predator/omnivore.

Electronic supplements S1. Mean abundance of identified nematode genera in sediments from: field site, control at day 0, control and treatments (medium and high) at different time (4, 15, 30 days). Also, total nematode abundance by microcosm is shown. Code of treatments: 0= T0, C= control, M= medium and H= high. FT= Feeding Type: 1A= selective deposit-feeder, 1B= non-selective deposit-feeder, 2A= epigrowth feeder and 2B= omnivore/predator. Hyphen indicates absence.

	F.T	C4	C15	C30	M4	M15	M30	H4	H15	H30	T0	FIELD
Anonchus	2A	11 ± 3	10 ± 2	8 ± 3	6 ± 3	8 ± 3	2 ± 1	7 ± 3	7 ± 3	3 ± 2	15 ± 9	8 ± 4
Anoplostoma	1B	3 ± 3	2 ± 1	2 ± 0	3 ± 2	1 ± 1	-	3 ± 2	2 ± 1	0 ± 1	3 ± 4	12 ± 8
Antomicron	1A	1 ± 1	1 ± 1	0 ± 1	1 ± 1	1 ± 2	0 ± 1	-	-	0 ± 1	-	-
Daptonema	1B	0 ± 1	1 ± 1	-	0 ± 1	1 ± 1	-	1 ± 1	-	-	1 ± 1	1 ± 1
Halalaimus	1A	-	-	-	0 ± 1	-	-	-	-	-	-	-
Kosswigonema	2B	0 ± 1	-	-	-	-	-	0 ± 1	-	-	0 ± 1	3 ± 5
Leptolaimus	1A	2 ± 1	2 ± 2	1 ± 1	1 ± 1	2 ± 2	1 ± 1	2 ± 1	1 ± 1	1 ± 1	3 ± 1	4 ± 5
Morphotype 3	1B	0 ± 1	-	-	-	-	-	-	-	-	-	-
Neochromadora	2A	-	-	0 ± 1	-	-	-	-	-	-	0 ± 1	0 ± 1
Oncholaimellus	2B	0 ± 1	-	-	-	1 ± 1	-	-	-	-	-	-
Oncholaimus	2B	0 ± 1	-	-	-	0 ± 1	-	0 ± 1	-	-	-	1 ± 1
Oxystomina	1A	7 ± 4	7 ± 3	9 ± 3	7 ± 4	6 ± 2	3 ± 2	4 ± 2	5 ± 4	6 ± 2	7 ± 4	6 ± 6
Pseudochromadora	2A	37 ± 12	48 ± 3	44 ± 15	45 ± 6	53 ± 14	87 ± 8	35 ± 10	53 ± 8	69 ± 11	26 ± 19	24 ± 17
Paradontophora	2B	2 ± 1	1 ± 2	2 ± 1	3 ± 1	1 ± 1	0 ± 1	4 ± 2	2 ± 1	1 ± 1	5 ± 3	3 ± 2
Paralinhomoeus	1B	3 ± 2	2 ± 1	3 ± 2	3 ± 2	1 ± 1	2 ± 3	5 ± 3	4 ± 2	3 ± 1	4 ± 4	8 ± 6
Sabatieria	1B	9 ± 2	5 ± 2	8 ± 2	7 ± 2	8 ± 4	1 ± 1	9 ± 3	6 ± 5	1 ± 1	8 ± 4	4 ± 3
Theristus	1B	1 ± 2	1 ± 1	1 ± 1	1 ± 1	0 ± 1	0 ± 1	-	0 ± 1	0 ± 1	3 ± 3	2 ± 4
Terschellingia	1A	16 ± 6	16 ± 4	20 ± 7	17 ± 1	19 ± 7	7 ± 5	20 ± 5	17 ± 9	15 ± 8	15 ± 8	17 ± 11
Viscosia	2B	10 ± 3	8 ± 4	4 ± 2	8 ± 2	2 ± 2	1 ± 1	12 ± 4	7 ± 4	2 ± 1	22 ± 13	22 ± 18
Nematodes total abundance	-	1514 ± 1374	1162 ± 489	937 ± 633	750 ± 402	709 ± 389	289 ± 101	249 ± 98	666 ± 229	430 ± 137	627 ± 96	780 ± 327

Electronic supplement S2. Post Hoc comparisons using the Tukey test:

A) PERMANOVA * significant difference

Time (days)	Grups	t	p(perm)	perm	p(MC)
4	C, M	0.96	0.5498	126	0.4745
	C, H	0.99986	0.4115	126	0.396
	M, H	0.99247	0.5061	126	0.4282
15	C, M	1.262	0.1302	126	0.1764
	C, H	1.2364	0.1066	126	0.2
	M, H	1.599	0.0085	126	0.050*
30	C, M	3.7808	0.0077	126	0.0006*
	C, H	2.4845	0.0077	126	0.006*
	M, H	1.3853	0.0992	126	0.1449

B) Two-way crossed ANOVA (all variables except oxygen). Repeated measures ANOVA by oxygen.

B1) TREATMENT Were, treatments: control (C), medium (M) and high (H) * Significant difference

OM	PHEOPIG	LIP
C=M 0.851699	C<M 0.000149*	C<M 0.010469*
C<H 0.000798*	C<H 0.000149*	C<H 0.000968*
M<H 0.002358*	M<H 0.000271*	M=H 0.503104
MS = 1.2644, df = 18	MS = 0.01386, df = 18	MS = 0.09640, df = 18

B2) TIME. Were, time in days: 4 (T4), 15 (T15) and 30 (T30). * Significant difference

Chl-a	PHEOPIG
T4<T30 0.003656*	T4<T15 0.020450*
T4=T15 0.411820	T4<T30 0.000156*
T15=T30 0.055659	T15<T30 0.006543*
MS= 3.2304 df = 18	MS = 0.01386 df = 18

B3) TREATMENT X TIME. Were letter represent the treatment and the number the time in days e.g. M0= medium at 0 days, significance $p<0.001$

Chl-a	PRT	O_2 T_4	O_2 T_{15}	O_2 T_{30}
C4<M4, H4, M15, H15, M30, H30	C4<H4, M15, H15, M30, H30	C0<C1 C0>M0, M1, M2, M3, M4, H0, H1, H2, H3, H4	C0>M1-M3, M5-M12, H1-H14	C0>M1-M10, M20, H1-H20
M4>C30, H30	M4<M15, H15, M30, H30	C1> M0, M1, M2, M3, M4, H1, H2, H3,H4	C1<C5, C11,C12 C1>MO-M14, HO-H14	C1> C5-C7, C9, C10, C12,C13, C15,C18,C20, M0-M15, M18, M20, H0-H22, H27
H4>C15, C30, H30	H4>C30 H4<H30	C2>M0, M1, M2, M3, M4, H1, H2, H3, H4	C2>M0-M13, H0-H14	C2, C3, C4> C20, M0-M13, M20, H0-H22
C15<M15, H15, M30, H30	C15<M15, H15, M30, H30	C3>M0, M1, M2, M3, M4, H1, H2, H3, H4	C3, C4>M0-M3, M5-M13, H0-H14	C5> M1-M9, M20, H1-H17, H20
M15>C30, H30	M15>C30	C4> M0, M1, M2, M3, M4, H1, H2, H3, H4	C5,C6, C7>M1-M3, M5-M7, M10, M11, H1-H14	C6> M1-M10, M20, H1-H17, H20
H15>C30, H30	H15>C30	M0>M1, M2, M3, M4, H1, H2, H3,H4	C8, C9> M1-M3, M5-M11, H1-H14	C7> M0-M10, M20, H1-H17, H20

Chl-a	**PRT**	**O_2 T_4**	**O_2 T_{15}**	**O_2 T_{30}**
C30<M30, H30	C30<M30, H30	M1<M4, H0	C10, C11, C12, C13> M1-M3, M5-M7, M10, M11, H1-H14	C9, C10,C12, C15> M1-M11, M21, H1-H18, H21
		M2<H0	C14> M1-M3, M5-M12,H1-H14	C11, C13, C14> M1-M10, M21, H1-H18, H21
		M3<M4, H0	M0> M1, M2, M5-M7, H1-H9, H11-H13	C16, C17, C18, C19 > M1-M11, M21, H1-18, H21
		M4<H0	M1< M4, M9-M14, H0	C20> M1-M11, M21, H1-H21
		M4>H1, H2, H3, H4		
		H0> H1, H2, H3, H4	M2< M3-M4, M9-M14, H0	C21<C25, C28
			M3<M4, M14, H2, H4	C22>M1-M11, M21, H1-H13, H21
			M4> M5-M7, H1- H14	C23, C25> M1-M13, M21, H0-H22
			M5< M9, M11-M14, H0	C24> M1-M13, M21, H0-H21
			M6, M7<, M9, M12-M14, H0	C26> M1-M12, M21, H0-H21
			M9< H1-H7, H11	C27> M1-M11, M21, H1-H21
			M10< M14	C28> M0-M13, M21, H0-H23
			M10> H2, H4	
			M11<M14	M0>M1-M9, M21, H1-H12, H21
			M11>H2,H4,H6	
			M12> H1-H7, H11	M1<M12-M20, M22-M28, H22-H28
			M13, H0> H1-H13	M2<M11-M20, M22-M28, H19, H22-H28
			M14> M10, M11, H1-H14	M3<M12-M20, M22-M28, H0, H22-H28
			H2, H4< H14	M4<M14-M20, M22-M28, H24-H28

Chl-a	PRT	O_2 T_4	O_2 T_{15}	O_2 T_{30}
				M5<M11-M20, M22-M28, H0, H12, H20, H22-H28 M6<M12-M20, M22-M28, H0, H22-H28 M7<M11-M20, M22-M28, H0, H19, H22-H28 M9< M12-M20, M22-M28, H0, H23-H28 M10< M15-M20, M22-M28, H24-H27 M11<M15-M18, M20, M22-M28, H3, H25, H26 M12<M23, M25, M26, M28 M12, M13> H1-H11 M13, M12, M14>H21 M13<M23, M25,M26, M28 M14> H1-H13 M15, M16> H1-H15, H17 M17>H1-H18 M18>H1-H17 M19>H1-H14 M20>H1-H17 M21<M22-M28, H24-H28 M14, M15, M16, M17, M19, M18, M20> M21, H21 M22> H1-H18, H21 M23> H1-H21 M24>H1-H18, H21

Chl-a	PRT	O_2 T_4	O_2 T_{15}	O_2 T_{30}
				M25, M26, M28> H1-H21 M27>H1-H18, H21 H0>H1-H14, H21 H0<H25, H26 H1<H16, H18, H19, H20, H22, H28 H2< H15, H16, H18-H20, H22-H28 H3<H15-H20, H22-H28 H4<H15, H16, H18-H20, H22-H28 H5, H6< H16, H18-H20, H22-H28 H7<H18-H20, H22-H28 H9<H16, H18-H20, H22-H28 H10, H11<H19, H22-H28 H12,H13,H14< H22-H28 H15, H17<H23-H28 H16, H18< H24-H28 H19>H21 H19, H20< H24, H25, H26 H22<H25, H26
MS=3.2304 df = 18	MS=0.11054 df = 18	MS = 0.90073 df = 60	MS = 1.3681 df = 168	MS = 1.6451 df = 77