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

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

3 Noelia Kandravicius\*<sup>1</sup>, Catalina Pastor de Ward<sup>2</sup>, Natalia Venturini<sup>3</sup>, Luis Giménez<sup>4</sup>,  
4 Marcel Rodriguez<sup>1</sup> & Pablo Muniz<sup>1</sup>.

5 1 Oceanografía & Ecología Marina, Instituto de Ecología y Ciencias Ambientales,  
6 Facultad de Ciencias, Universidad de la República (UdelaR), Iguá 4225, Montevideo,  
7 11400, Uruguay

8 2 Instituto de Diversidad y Evolución Austral (IDEAUs), CCT, CONICET, Bulevar  
9 Almirante Brown 2915, Puerto Madryn, Chubut, Argentina

10 3 Laboratorio de Biogeoquímica Marina, Oceanografía & Ecología Marina, Instituto de  
11 Ecología y Ciencias Ambientales, Facultad de Ciencias, UdelaR, Iguá 4225, Montevideo,  
12 11400, Uruguay

13 4 School of Ocean Sciences, Bangor University, LL59 5AB, Menai Bridge, Anglesey,  
14 UK

15 [\\*nkandra@fcien.edu.uy](mailto:nkandra@fcien.edu.uy)

16  
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18 Rocha, Uruguay

19  
20 **Running page head:** Response of nematodes to organic enrichment.

21  
22 **Abstract**

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

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

## 42 **Introduction**

43 Nutrient enrichment of marine/estuarine areas can favour algal growth and lead to  
44 eutrophication, the occurrence of anoxia and hypoxia, fish-kills (Glasgow & Burkholder  
45 2000), loss or degradation of habitat for benthic organisms and a decrease in the number  
46 of fisheries. Eutrophication is considered one of the major stresses for aquatic  
47 environments, and it is characterized by excess biomass (Sampou & Oviatt 1991) and  
48 accumulation of refractory organic matter. Anthropogenic activities including agricultural  
49 production, industrial and domestic effluents, modify the physicochemical and biological  
50 conditions of estuarine systems (Day et al. 1989, Perissinotto et al. 2010). These activities  
51 generally intensify the process of eutrophication introducing inorganic nutrients and the  
52 consequent increase in algal biomass and primary productivity in the water column  
53 (Cloern 2001, Pusceddu et al. 2009).

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

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

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

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

91 Here, we quantified the effects of organic enrichment on taxonomic and functional  
92 diversity of assemblages of free-living nematodes from Rocha Lagoon (Atlantic coast of  
93 Uruguay, South America). The process of eutrophication existing in Rocha lagoon (see  
94 “study area” in “material and methods” for details) is representative of the situation being  
95 experienced by other coastal systems worldwide (Cloern 2001). We studied the effects of  
96 enrichment, through a laboratory experiments, using nematode assemblages as a model  
97 system. Laboratory experiments are considered an appropriate approach to study the  
98 effect of organic enrichment in marine and estuarine communities (Coull & Chandler  
99 1992). Microcosm experiments enable the establishment of cause-effect relationships  
100 (Nilsson et al. 1991) and can be used to determine which organism are indicators of  
101 disturbances (Heip et al. 1985, Coull 1988).

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

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

127

## 128 **Materials and methods**

129

### 130 **Environmental set up**

131 Experimental surface sediments and experimental nematodes were collected during  
132 January 2015 in the south of Laguna de Rocha, Uruguay (34°39'47.42''S,  
133 54°13'47.36''W, see Figure 1 Kandratavicius et al 2015). Rocha Lagoon is a choked type  
134 lagoon (Kjerfve & Magill 1989, Conde et al. 2000) with an area of 7304 ha, shallow and

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

138

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

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

150

#### 151 **Sampling and microcosm set up**

152 Sediment samples and fauna were taken by hand because of the shallow habitats (<1m)  
153 in a location known as “old bar” (34°39’47.20’’S, 54°13’50.41’’W) characterized by fine  
154 sediments (69% mud, fine and medium sand), low organic content (~1.32%), 18.9 of  
155 salinity (average from summer season) and high temperatures (28 °C) (Giménez et al.  
156 2014, Kandratavicius et al. 2015). Five plastic cores (2.7 cm internal diameter) were taken  
157 to 10 cm depth in the sediment for the description of the community structure and three  
158 surface sediment samples (1cm depth, approximately 300 g) for the estimation of total  
159 organic matter, chlorophyll a and organic biopolymers (total lipids, carbohydrates and  
160 proteins). All of these samples were considered as field control.

161

162 A key point for the validity of the experimental study is that it should be as homogeneous  
163 as possible across experimental units and the effects of treatments must be stronger than  
164 the “microcosm effect”, i.e. the effect of manipulation of sediments (Austen & McEvoy  
165 1997). We carefully collected approximately 15 litres of surface sediment to set up the  
166 experimental units or microcosms. The fresh sediment collected in the lagoon was  
167 transported to the laboratory, stored in two containers with aeration for approximately 24  
168 hours. Thereafter, the sediment was gently homogenized with a spoon and five random

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

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

192 At the beginning of the experiment (time =  $T_0$ ), five microcosms of the control treatment  
193 were used to analyze the structure of nematode community (microcosms are destroyed  
194 during the sampling and thus are used only once). At times of 4, 15 and 30 days, five  
195 microcosms of each treatment were used to analyze the structure of the nematodes  
196 community and three microcosms were used to analyze the organic matter, biopolymers  
197 and Chl-a content (Fig. 1). The dissolved oxygen concentration and temperature were  
198 measured daily in the water matrix (measurements done with a O<sub>2</sub> microsensor Unisens®  
199 OX50 and YSI® multi-parameter respectively).

200

## 201 **Sample processing**

202 The content of each microcosm was used to analyze different attributes of sediment.  
203 Photosynthetic pigments (Chl-a and phaeopigments) were analyzed according to  
204 Lorenzen (1967), modified by Sündback (1983) for sediments. Total organic matter (OM)  
205 was analyzed based on Byers et al. (1978) and expressed as percentage (%). Biochemical  
206 composition of organic matter was analyzed following the protocols described in  
207 Danovaro (2010). Total protein (PRT) analysis was conducted according to Hartree  
208 (1972) modified by Rice (1982) to compensate for phenol interference. Total  
209 carbohydrates (CHO) were analyzed according to Gerchacov & Hatcher (1972). Total  
210 lipids (LIP) were extracted by ultrasonication with a mixture of chloroform: methanol  
211 (1:2 v/v) and analyzed following the protocol described in Marsh & Weinstein (1966).  
212 Blanks for each analysis were performed with pre-combusted sediment (450°C, 4 hrs.).  
213 PRT, CHO and LIP concentrations were expressed as bovine serum albumin, glucose and  
214 tripalmitine equivalents, respectively. Protein, carbohydrate and lipid concentrations  
215 were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75  
216 µg, respectively (Fabiano & Danovaro 1994). The sum of protein, lipid and carbohydrate  
217 carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable  
218 estimate of the labile fraction of organic matter (Fabiano et al. 1995) and to classify the  
219 trophic status of the sediments. Also, the protein to carbohydrate ratio (PRT : CHO) and  
220 the carbohydrate to lipid ratio (CHO : LIP) were calculated and used as indicators of the  
221 status of biochemical degradation processes (Galois et al. 2000).

222 In order to sample nematodes, the content of each microcosm was washed between a 500  
223 µm sieve and 63 µm one using filtered water. To extract the meiofauna from the sediment  
224 fraction, retained on the sieve of 63 µm we applied a flotation technique using Ludox HS  
225 40 Coloidal Silica ( $1.18 \text{ g cm}^{-3}$ ) and centrifugation (Heip et al. 1985, Vincx 1996). This  
226 process was repeated 3 times whereby each time the supernatant Ludox containing the  
227 meiofauna organisms was decanted and washed. The final washed and extracted sample  
228 was then preserved in 4% formaldehyde, and a small amount of Rose Bengal was added  
229 to facilitate the identification. In binocular loupe 100 nematodes were randomly picked  
230 out of each microcosm and mounted on glass slides for genus identification under  
231 microscope (Sommerfield & Warwick, 1996) using pictorial keys (Platt & Warwick 1983,  
232 1988, Warwick et al. 1998).

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



236

### 237 **Structure of nematode assemblages and biological/functional traits**

238 Richness (as number of genera) and abundance of nematodes per genera was determined  
239 for each microcosm. Each one was classified according to their life strategy into a scale  
240 of coloniser/persister (c-p score: Bongers 1990, Bongers et al. 1991). The scale range is  
241 defined from extreme colonisers (cp score = 1) to extreme persisters (c-p score= 5). The  
242 maturity index (MI) of the community was calculated using the formula (Bongers et al.  
243 1991):

$$244 \quad MI = \sum (v(i) \times f(i))$$

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

246 Additionally nematode genera were assigned to feeding types according to Wieser's  
247 (1953) classification based on the morphology of buccal cavity: selective deposit-feeder  
248 (1A), nonselective deposit-feeder (1B), epigrowth feeder (2A) and omnivore/predator  
249 (2B). This classification was used to calculate the Index of Trophic Diversity (Heip et al.  
250 1985), calculated as:

$$251 \quad ITD = \sum \theta^2$$

252 where  $\theta$  is the percentage contribution of each trophic group according to Wieser (1953).  
253 ITD values vary in a range between 0.25 (high trophic diversity: the four groups have a  
254 representation of 25%) and 1.0 (low trophic diversity: a single trophic group dominates,  
255 100%).

256

### 257 **Data analysis**

258 Multi and univariate techniques were used for data analysis using the software PRIMER  
259 6.0.2 (Clarke & Gorley 2006) and STATISTICA 10.0 from StatSoft. If needed, data were  
260 transformed and re-checked to determine if parametric assumptions were applicable.  
261 Comparisons to test changes in biota and trophic status of sediment (based on organic  
262 matter, chlorophyll a, phaeopigments, and biopolymers) between control groups were  
263 done in order to assess the "microcosm effect" (abiotic and biotic changes due experiment  
264 artifacts) using a one-way ANOVA with five levels: field control, time 0 (microcosm  
265 control), controls at time 4, 15 and 30. If the differences in ANOVA were significant, two  
266 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<sub>0</sub> vs. controls to 4 days, 15 and 30 (C<sub>4</sub>, C<sub>15</sub>, C<sub>30</sub>) 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 T<sub>0</sub> 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 T<sub>0</sub> 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<sub>0</sub> and  
297 controls, while in the treatments recorded values were  $> 1$ . Carbohydrates to lipids ratio  
298 (CHO/LIP) showed values  $\gg 1$  in Field, T<sub>0</sub>, controls and both treatments.

## 299 Enrichment

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

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

311 The addition of *S. platensis* resulted in a significant decrease of dissolved oxygen (Table  
312 3, Fig. 4). Its levels reached the limit of hypoxia (2 mg l<sup>-1</sup>) after ~ 1 day and tended to  
313 recover after around 10 days; recovery took place more slowly after addition of *S.*  
314 *platensis* at the highest treatment (Fig. 4, Electronic supplement S2).

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

320

## 321 **Responses of the nematode assemblage**

322 A comparison of univariate measures of assemblages between field and control samples  
323 at day 0 indicates how much the assemblages in the experimental conditions mimic those  
324 of the natural environment (Fig. 5). In general, nematode assemblages were similar in  
325 field, control microcosms and T<sub>0</sub>. There were no significant differences in the log-  
326 transformed number of nematodes (F<sub>4,20</sub>=1.641 p > 0.05). Also, nematodes abundance  
327 did not vary significantly at the end of the experiment in comparison with T<sub>0</sub>.

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

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

337 The number of genera (richness) was significantly different among treatments, with lower  
338 values in medium and high treatment respect to control. There were no significant  
339 changes over time in the genera richness in the controls, but in the treatments, both the  
340 medium and high treatment suffered a decrease in the number of genera over time (Table  
341 4).

342 There was a significant multivariate effect of enrichment and time on the nematode  
343 assemblage (PERMANOVA significant Time x Treatments interaction: Pseudo-  $F=$   
344 2.735, Monte-Carlo  $p = 0.0006$ ; 9920 permutations), showing that differences among  
345 treatments depend on the sampling time (Table 5 and Electronic supplement S2). After  
346 15 days, significant differences were found only between medium and high enrichment  
347 treatments ( $t = 1.599$ , Monte-Carlo  $p = 0.05$ , 126 permutations); however, after 30 days,  
348 differences between control versus both medium and high enrichment treatments were  
349 significant (Monte-Carlo  $p = 0.0006$ ,  $p = 0.006$ , 126 permutations) (Table 5 and Electronic  
350 supplement S2).

351 The SIMPER procedure indicated that the same genera contributed to similarity within  
352 groups of controls at 4, 15 and 30 days: *Pseudochromadora* and *Terschellingia*. In  
353 addition, the average of dissimilarity between controls at different time did not exceed 20  
354 %. The pair-wise comparisons between T0 and field showed also a significance  
355 difference, but the R value was low ( $R = 0.28$ ,  $p = 0.04$ , 126 perm.), suggesting a small  
356 effect (Fig. 8, average of dissimilarity: 38%). The most marked differences (average of  
357 dissimilarity 50%) were observed in the pair-wise comparisons between enriched  
358 treatments at 4 days and 30 days.

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

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

369

#### 370 Community maturity and trophic diversity

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

382

#### 383 **Discussion**

384 In assessing the effect of enrichment on nematode assemblages we used a microcosm  
385 approach. We therefore increased our capacity to establish cause-effect relationships at  
386 the cost of losing realism. We minimized effects related to the construction of the  
387 laboratory assemblages, the so-called microcosm effect (leading to microhabitat  
388 homogenization temporal hypoxia and mortality of sensitive species): at T<sub>0</sub> assemblages  
389 did not differ from those in the field (Fig. 9) and both field conditions and the control at  
390 the beginning of the experiment had similarly aged and degraded sedimentary organic  
391 matter (PRT : CHO < 1: Dell'Anno et al. 2002), and low quality organic matter (CHO :  
392 LIP >>1). The fact that the enrichment effects were stronger than the effects of sediment  
393 disruption (see Table 1), validated our approach according to the criteria stated in Austen  
394 & McEvoy (1997). The environmental variables and the nematode assemblages varied  
395 little in the controls. The little variation in the nematode assemblage between field, T<sub>0</sub>

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

406 The experimental treatments were accurate in recreating organic enrichment and its  
407 consequences, in terms of trophic status of sediment and hypoxia. In our experiment we  
408 simulated an important input of labile organic matter with the addition of *Spirulina*  
409 *platensis*. Hence, as particles sank in the experimental units, there was an increase in Chl-  
410 a and proteins (detected as a significant interaction between enrichment by time. Table  
411 2). There was also an increment in phaeopigments four days after the beginning of the  
412 experiment. There was also a change in the age of the organic matter, from aged to  
413 live/fresh, as quantified from the ratio of proteins to carbohydrates (PRT : CHO < 1 in  
414 controls and >1 in enriched treatments: Danovaro et al. 1993). Equally, the quality of  
415 organic matter increased as indicated from the increase in levels of BPC (Fabiano et al.  
416 1995) and low CHO : LIP ratio (Joseph et al. 2008) driven by an increase in the  
417 concentration of lipids. The values of CHO : LIP (>>1) indicated that that the input of  
418 fresh organic matter had a low nutritional level albeit higher than the registered in  
419 controls. Enrichment also resulted in sediment hypoxia (oxygen concentrations < 2.8 mg  
420 l<sup>-1</sup>: Diaz & Rosenberg 1995) in consistence with previous studies (Armenteros et al.  
421 2010).The magnitude by hypoxia was higher over the first 15 day of the experiment  
422 (detected as significant interaction enrichment by time: Table 2). By contrast, most  
423 environmental variables varied little over time in the controls, with the exception of  
424 increases in organic matter and phaeopigments, but we expected such patterns, as over  
425 time, particles would sink slowly from the water column.

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

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

439 Most of the genera were affected by enrichment, but some responses were difficult to  
440 interpret and may reflect non-linear or complex responses to the multiple environmental  
441 changes associated to enrichment (e.g. changes in dissolved oxygen and organic matter  
442 composition). For instance, *Anoplostoma*, which decreased in abundance in both  
443 enrichment treatments, has been reported as favoured by organic enrichment (Kapusta et  
444 al. 2006). *Viscosia* composed mainly of facultative predators, able to exploit a wide range  
445 of food resources (Moens & Vincx 1997), had the lowest abundance at medium levels of  
446 enrichment and higher abundance at the high level of enrichment. The increase in density  
447 of *Pseudochromadora* in response to organic enrichment is more logical: these are  
448 epistrate feeders and benefit by the availability and diversity of food resources (Pinto &  
449 Bemvenuti 2003, Kapusta et al. 2006). Some genera (*Oxystomina*, *Sabatieria*,  
450 *Terschellingia* and *Paradontophora*) appeared to be tolerant to enrichment, as they did  
451 not show changes among treatments. This is consistent with previous studies showing  
452 that such genera are well known for their proliferation in stressful conditions or in close  
453 association with sediment organic enrichment (Mirto et al. 2002). Species of the genus  
454 *Terschellingia* are tolerant to a diversity of stressors in soft bottoms (Schratzberger et al.  
455 2006); *Sabatieria* and *Oxystomina* are tolerant to aquaculture deposition; *Sabatieria* is  
456 well adapted to live in environments with high organic carbon loads, low oxygen, and  
457 high sulphide concentrations (Jensen et al. 1992, Soetaert & Heip 1995). *Parodontophora*  
458 species have been reported to be unresponsive to changes in chl-a sediment  
459 concentrations (Quang et al. 2016).

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

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

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

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



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

501 Given the multiple stressor nature of enrichment (organic matter content and quality is  
502 increased, but oxygen levels drop and driving the increases in concentrations of hydrogen  
503 sulfide and ammonia), we cannot identify which stressor drives the observed patterns in  
504 nematodes assemblages. Decreases in abundance may be driven mainly by hypoxia, as  
505 suggested by Gray et al. (2002) and Van Colen et al. (2009). Oxygen limitation is also  
506 suggested by the fact that the less responsive trophic groups were those characterized by  
507 low metabolic rates. Behavioral and physiological adaptations (e.g. migration to “oxygen  
508 islands”: Balsamo et al. 2012; slow movement and low metabolic rates: Warwick & Price  
509 1979, Warwick & Gee 1984) may explain why some groups did not were affected their  
510 abundances (or maintain their densities) In addition, tolerance hypoxia would have allow  
511 the access to organic matter in increased amount and quality (availability of food), which  
512 are key controlling factors of the growth, metabolism and distribution of benthic  
513 communities within the substrate (Danovaro & Fabiano 1997, Venturini et al. 2011).

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

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

530

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540

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794

795 **TABLES**

796 **Table 1.** Results of least square means (t-test) (1) field vs. T<sub>0</sub> to test the differences  
797 between field and the experimental conditions; (2) T<sub>0</sub> 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.

	<b>Field vs T0</b>	<b>T0 vs C4</b>	<b>T0 vs C15</b>	<b>T0 vs C30</b>
<b>OM</b>	t4=1.37, 0.242	t4=-1.11, 0.327	t4=6.45, 0.003*	t4=-2.81, 0.04*
<b>Chl-a</b>	t4= 3.35, 0.028*	t4=-4.22, 0.01*	t4=8.32,0.001*	t4=-1.04, 0.35
<b>Pheopig</b>	t4=4.63, 0.009*	t4=0.31, 0.77	t4=6.17, 0.003*	t4=-11.7, 0.0002*
<b>CHO</b>	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 (T<sub>0</sub> vs Field and T<sub>0</sub> 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.

<b>Increase (%)</b>	<b>Chl-a</b>	<b>Phaeopig</b>	<b>OM</b>	<b>CHO</b>	<b>PRT</b>	<b>LIP</b>	<b>BPC</b>
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 <sub>2,18</sub> )	Time (F <sub>2,18</sub> )	Treatment x Time (F <sub>4,18</sub> )
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 <sub>2</sub> Time 4	F <sub>(2, 12)</sub> = 529.34, p < 0.00001*	F <sub>(4, 48)</sub> = 24.545, p < 0.00001*	F <sub>(8, 48)</sub> = 19.607, p < 0.00001*
Time 15	F <sub>(2, 12)</sub> = 171.82, p < 0.00001*	F <sub>(14, 168)</sub> = 11.779, p < 0.00001*	F <sub>(28, 168)</sub> = 8.921, p < 0.00001*
Time 30	F <sub>(2, 12)</sub> = 53.542, p < 0.00001*	F <sub>(27, 324)</sub> = 46.642, p < 0.00001*	F <sub>(54, 324)</sub> = 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 <sub>2,36</sub> )	Time (F <sub>2,36</sub> )	Treatment x Time (F <sub>4,36</sub> )	Field vs To (F <sub>1,8</sub> )
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

818

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

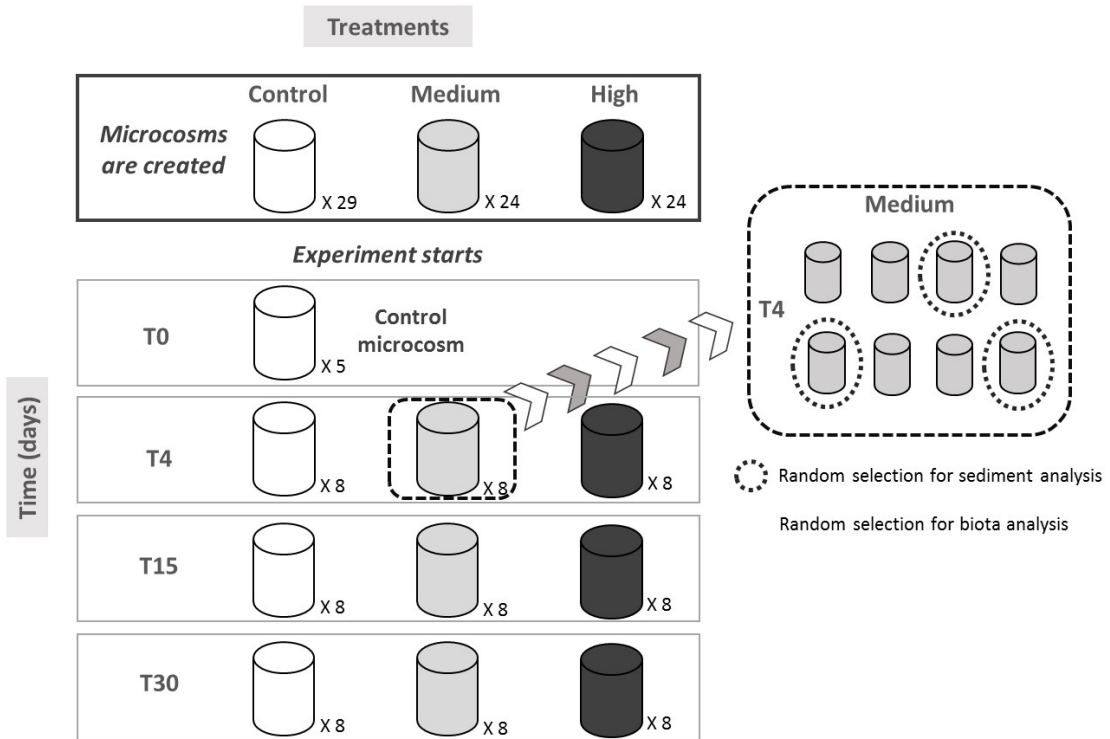
	df	SS	MS	Pseudo-F	P (Montecarlo)	perm.	p(perm)
<b>Treatment</b>	2	1799	899.5	5.5485	0.0001*	9936	0.0001*
<b>Time</b>	2	3394.5	1697.2	10.469	0.0001*	9930	0.0001*
<b>Treatment x Time</b>	4	1773.8	443.45	2.7354	0.0006*	9920	0.0003*
<b>Residual</b>	36	5836.2	162.12				
<b>Total</b>	44	12803					

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

822

823 **FIGURES**



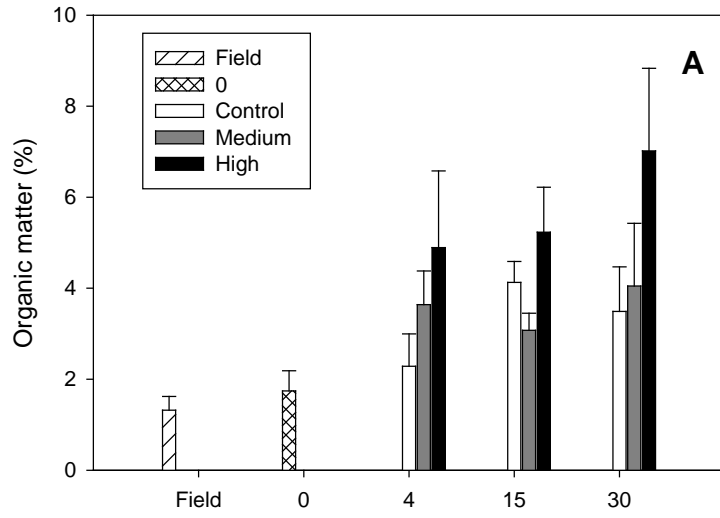
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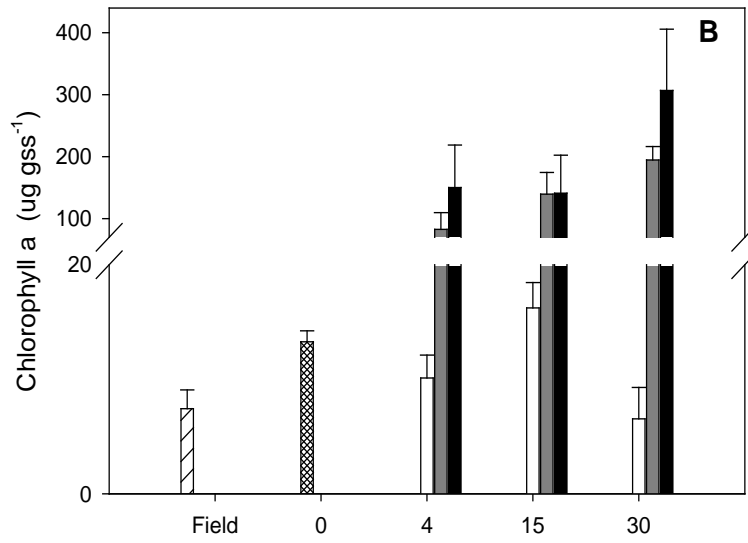
826 **Fig. 1.**

827

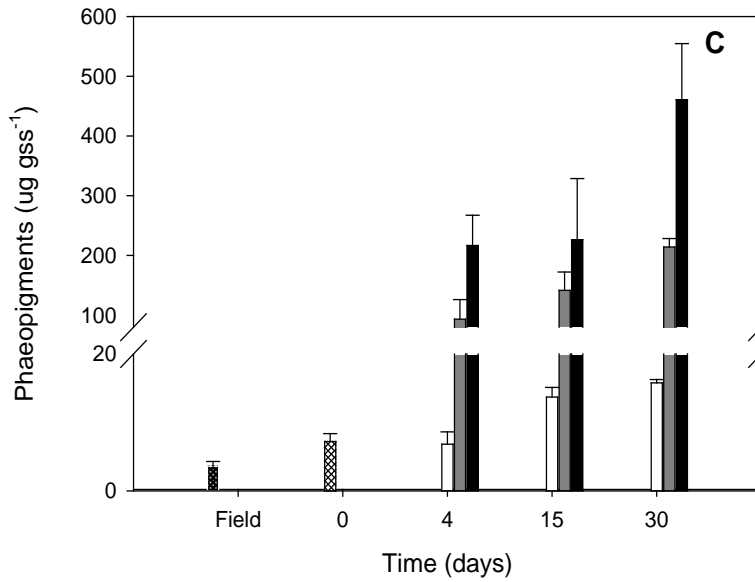
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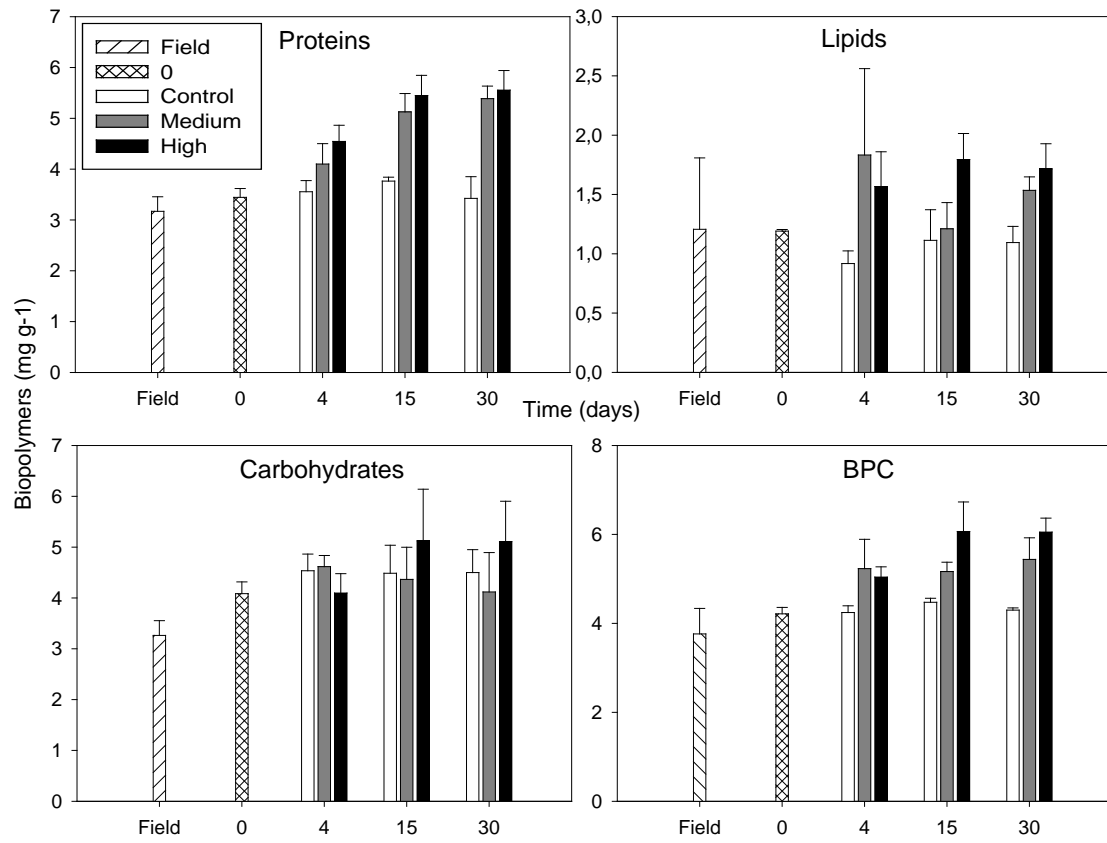
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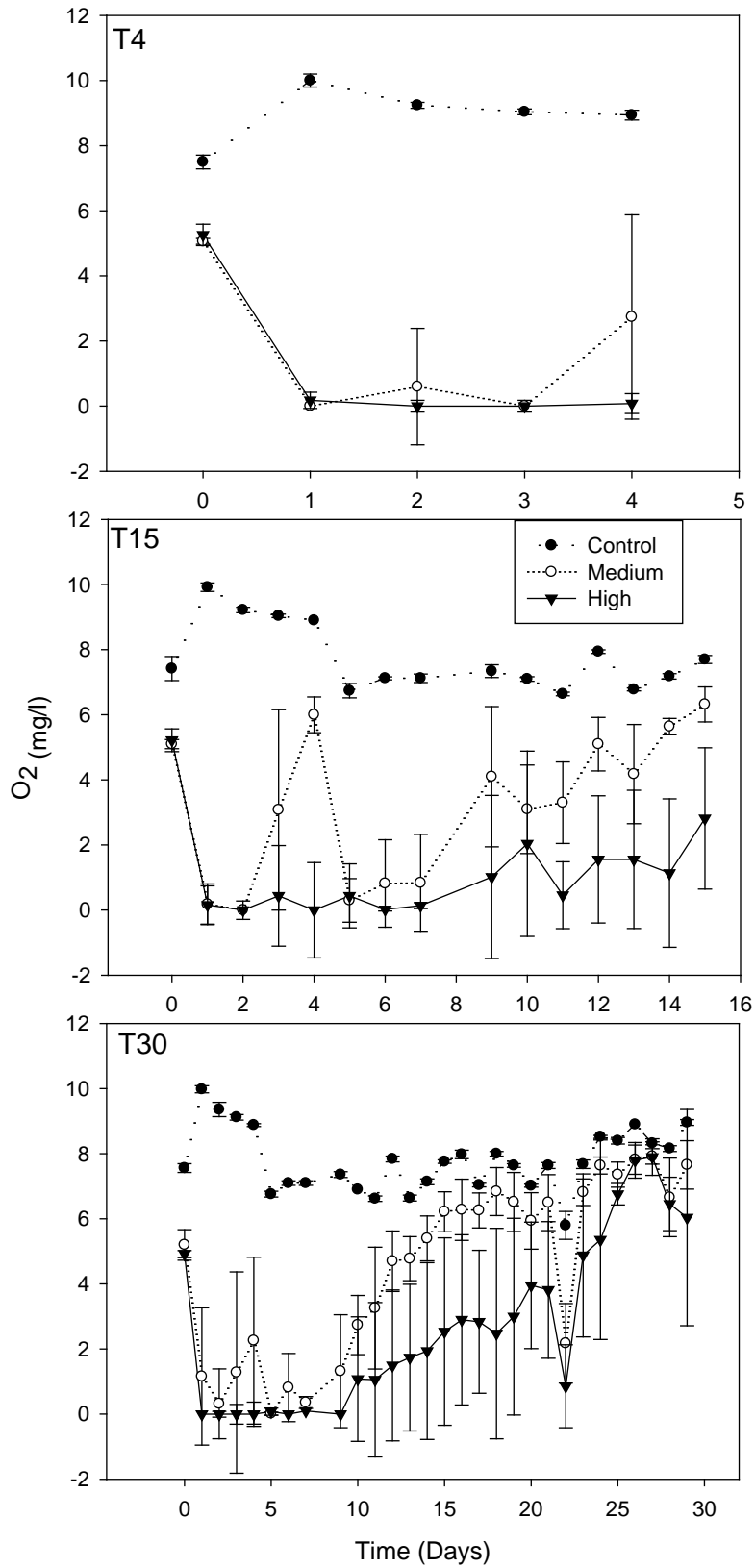


831 **Fig. 2.**



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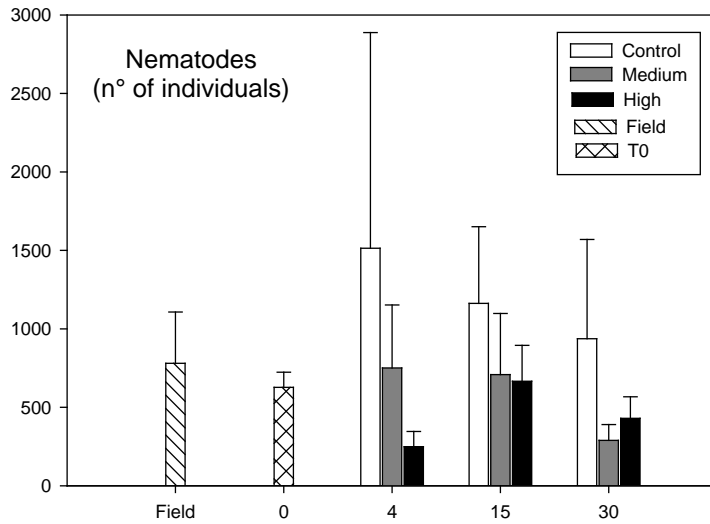
833 **Fig. 3.**



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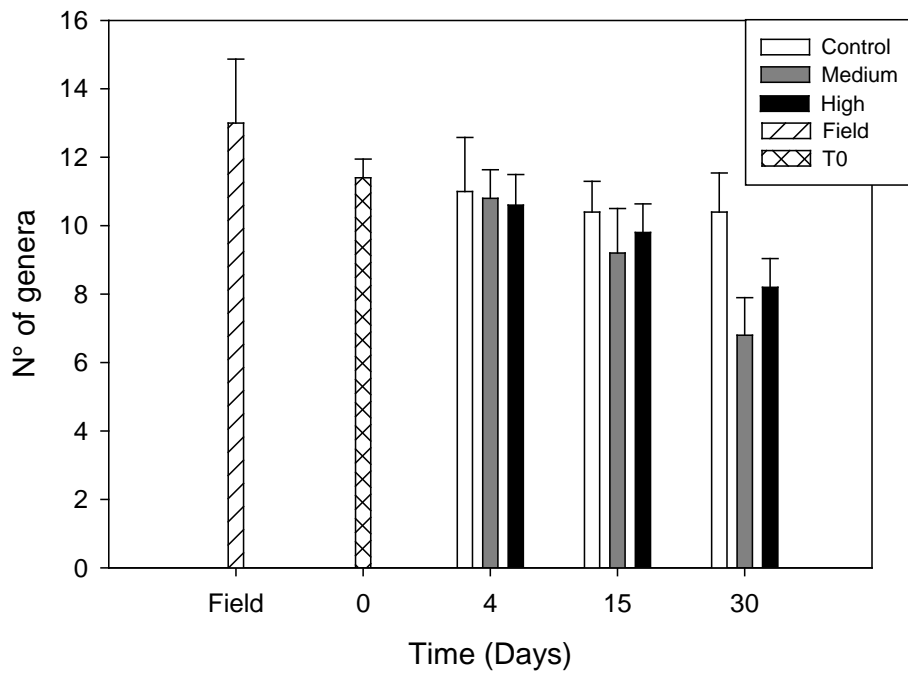
835 **Fig. 4.**





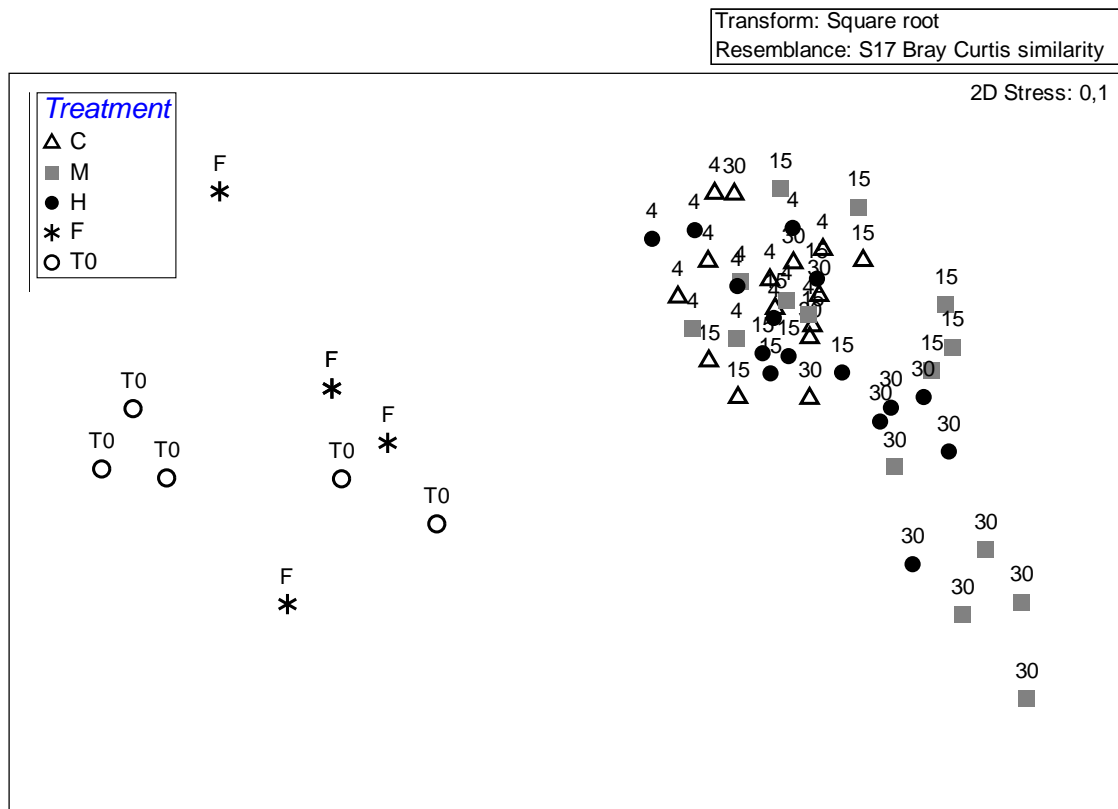
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837 **Fig. 5.**



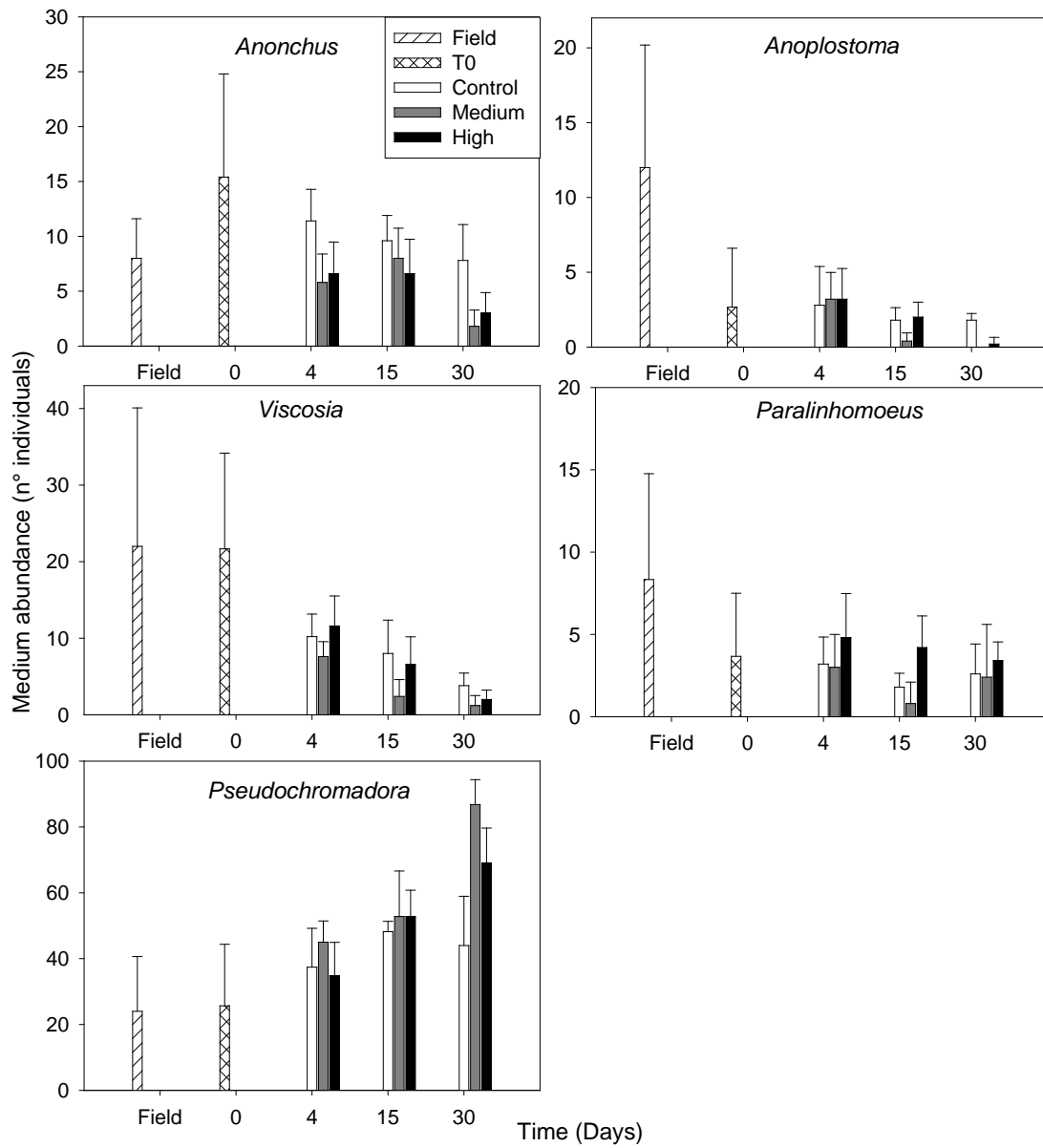
838

839 **Fig. 6.**



840

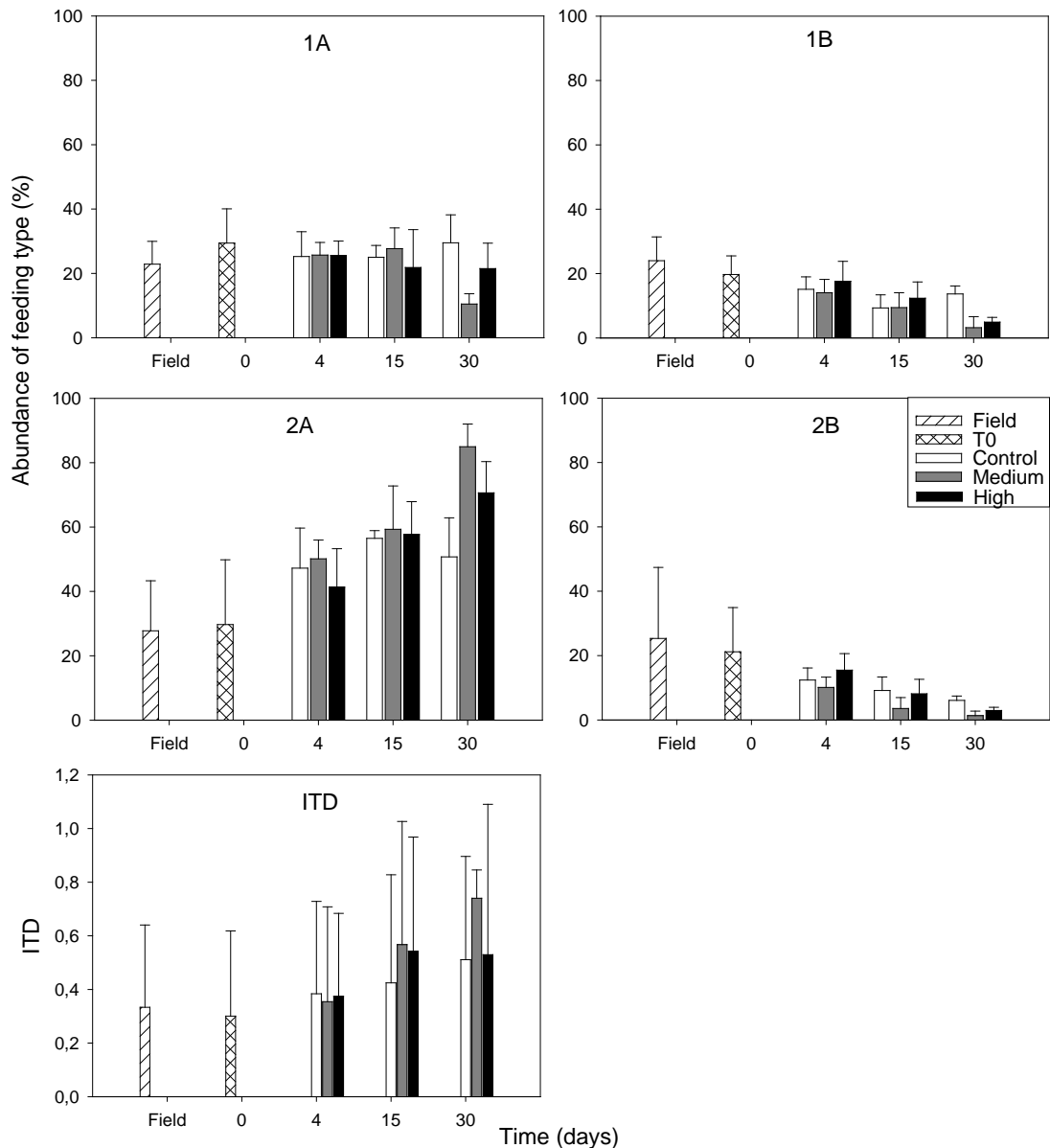
841 **Fig. 7.**



842

843

**Fig. 8.**



844

845 **Fig. 9.**

846 **CAPTIONS**

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

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

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

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

862 **Fig. 5.** Mean values and SD of abundance of nematodes measured from field site, Time  
863 0 and microcosms treatments (control, medium and high) at different time (4, 15 and 30  
864 days).

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

868 **Fig. 7. Non metric** multidimensional scaling ordination of samples based on square-  
869 root transformed data of density of nematode genera in sediment from: field (F), time  
870 0 (T0), control (C), medium treatment (M) and high treatment (H). Number upper symbol  
871 indicates days after the onset of the experiment.

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

874 **Fig. 9.** Average percentage of ITD value and feeding types of nematode assemblages in  
875 sediments from field site, control at T0, control and treatments at different time (4, 15 and  
876 30 days). Feeding types after Wieser (1953): 1A= selective deposit-feeder, 1B= non-  
877 selective deposit-feeder, 2A= epigrowth-feeder, 2B= predator/omnivore.

878

879 **Electronic supplements S1.** Mean abundance of identified nematode genera in  
880 sediments from: field site, control at day 0, control and treatments (medium and high) at  
881 different time (4, 15, 30 days). Also, total nematode abundance by microcosm is shown.  
882 Code of treatments: 0= T0, C= control, M= medium and H= high. FT= Feeding Type:  
883 1A= selective deposit-feeder, 1B= non-selective deposit-feeder, 2A= epigrowth feeder  
884 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

<b>Chl-a</b>	<b>PHEOPIG</b>
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. Where letter represent the treatment and the number the time in days e.g. M0= medium at 0 days, significance  $p<0.001$

<b>Chl-a</b>	<b>PRT</b>	<b>O<sub>2</sub> T<sub>4</sub></b>	<b>O<sub>2</sub> T<sub>15</sub></b>	<b>O<sub>2</sub> T<sub>30</sub></b>
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>M0-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



<b>Chl-a</b>	<b>PRT</b>	<b>O<sub>2</sub> T<sub>4</sub></b>	<b>O<sub>2</sub> T<sub>15</sub></b>	<b>O<sub>2</sub> T<sub>30</sub></b>
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 M4>H1, H2, H3, H4 H0> H1, H2, H3, H4	M1< M4, M9-M14, H0 M2< M3-M4, M9- M14, H0 M3<M4, M14, H2, H4 M4> M5-M7, H1- H14 M5< M9, M11-M14, H0 M6, M7<, M9, M12- M14, H0 M9< H1-H7, H11 M10< M14 M10> H2, H4 M11<M14 M11>H2,H4,H6 M12> H1-H7, H11 M13, H0> H1-H13 M14> M10, M11, H1- H14 H2, H4< H14	C20> M1-M11, M21, H1-H21 C21<C25, C28 C22>M1-M11, M21, H1-H13, H21 C23, C25> M1-M13, M21, H0-H22 C24> M1-M13, M21, H0-H21 C26> M1-M12, M21, H0-H21 C27> M1-M11, M21, H1-H21 C28> M0-M13, M21, H0-H23 M0>M1-M9, M21, H1- H12, H21 M1<M12-M20, M22- M28, H22-H28 M2<M11-M20, M22- M28, H19, H22-H28 M3<M12-M20, M22- M28, H0, H22-H28 M4<M14-M20, M22- M28, H24-H28

Chl-a	PRT	O <sub>2</sub> T <sub>4</sub>	O <sub>2</sub> T <sub>15</sub>	O <sub>2</sub> T <sub>30</sub>
				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 <sub>2</sub> T <sub>4</sub>	O <sub>2</sub> T <sub>15</sub>	O <sub>2</sub> T <sub>30</sub>
				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