Journal of Experimental Marine Biology and Ecology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Natural recovery of *Zostera noltii* seagrass beds and benthic nematode assemblage responses to physical disturbance caused by traditional harvesting activities

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ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form 20 February 2017
Accepted 8 March 2017
Available online xxxx

Keywords:
Sediment digging
Benthic nematodes
Extracellular enzymatic activity (EEA)
Zostera noltii
Natural habitat recovery
Field experiment

ABSTRACT

In the intertidal seagrass beds of *Zostera noltii* of Mira estuary (SW, Portugal) the harvesting practices are frequent. The traditional bivalve harvesting not only affects the target species as the remaining biological assemblages. The main aim of this study was to assess the disturbance caused by sediment digging in the recovery of the seagrass beds habitat, through an experimental fieldwork. The responses of the seagrass plant condition, the sediment microbial activity and the nematode assemblages were investigated after the digging activity in seagrass beds. A total of four experimental plots were randomly demarcated *in situ*, two plots were subjected to the disturbance - "Digging" - while other two were "Control"; the sampling occurred in five occasions, from May to October: T_0–before digging; T_1–14 days after digging; T_2–45 days; T_3–75 days; and T_4–175 days. The environmental variables measured in the sediment and the photosynthetic efficiency (α) of the *Z. noltii* plants in each plot and sampling occasion registered similar values, throughout the experiment. The extracellular enzymatic activity (EEA) clearly presented a temporal pattern, although no significant differences were obtained between digging and control plots. Nematode assemblages registered high densities, revealing the absence of the digging effect: control plots maintained similar density and diversity throughout the experiment, while the density and diversity between digging plots was significantly different at T_0 and T_4; the trophic composition was similar for both control and digging plots, characterized mainly by non-selective deposit feeders (1B) and epigrowth feeders (2A).Organic matter, nitrate and mean grain size explain a significant amount of the variation in the nematode genera composition. This study demonstrated the capacity of the seagrass habitat to recover under low intensity physical disturbance associated to harvesting.

1. Introduction

Seagrass beds comprise some of the most heterogeneous landscape structures of shallow-water estuarine/marine ecosystems in the world and are reported to be declining worldwide (Hughes et al., 2009). These beds have important ecological roles in coastal ecosystems providing high-value ecosystem services compared to other marine and terrestrial habitats. They are typically considered as ecosystem engineers playing an important role in structuring pelagic and benthic assemblages (Bos et al., 2007). Many studies reported seagrass beds as having higher biomass, abundance, diversity and productivity of benthic organisms than the unvegetated sediments (Boström et al., 2006; Fonseca et al., 2011; Orth et al., 2006). They are also effective carbon sinks in the biosphere (Duarte et al., 2010). Their high sensitivity to environmental deterioration and widespread geographical distribution make seagrasses useful as "miner's canaries" for the coastal deterioration (Marbà et al., 2006; Orth et al., 2006). Moreover, they are important habitats to a large set of fauna, providing nutrients, shelter against predators and nursery for the juveniles (Barbier et al., 2011; Orth et al., 2006).

Bivalve harvesting is a very common activity in European estuarine ecosystem (Carvalho et al., 2013; Johnson et al., 2007; Kaiser et al., 2001). In Portugal bivalve harvesting has a long tradition, with an estimated consumption rate *per capita* of 58.5 kg/year (Oliveira et al., 2013). While the traditional harvesting activities affect solely the targeted species, the digging of the sediments cause physical disturbances with effects on the remaining biological assemblages by

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http://dx.doi.org/10.1016/j.jembe.2017.03.003
0022-0981/

exposing benthic species to desiccation, to predators or burial and with the consequent removal of biogenic structures that are important for the oxygenation and stabilization of the sediments (Gutiérrez et al., 2004). The digging activity can lead to the migration of the benthic infauna to adjacent habitats less suitable for them or even to the complete defaunation due to physical damage or direct mortality (Ramsay and Kaiser, 1998).

The harvesting practices of bivalve molluscs for human consumption and polychaete worms for recreational fishing in intertidal seagrass beds of *Zostera noltii* are frequent and intense in Mira estuary, located in the Atlantic Coast of SW, Portugal. These seagrass beds were denser in the past, but nowadays the vegetation is in a natural recovery process after a major collapse in 2008, with still unknown causes. From 2009 onwards a non-uniform natural recovery was observed with some seagrass beds having high biomass, while others have very low biomass values (Materatski et al., 2015, 2016). This study provides the opportunity to investigate if the digging activity during the harvesting could have triggered and stimulated the habitat loss of the estuarine intertidal seagrass beds.

It is now widely accepted that marine nematodes are good indicators of environmental impacts in a variety of habitats, types of disturbance (*i.e.* organic, physical and chemical) and temporal scales (short to long-term disturbances); also the response of nematodes to disturbance were demonstrated to be complementary to other benthic communities (Patrício et al., 2012; Xu et al., 2014). Nematodes are great ecological flags due to the ubiquitous distribution (Austen and Widdicombe, 2006). Their temporal and spatial distributions are often determined by the ecosystem interactions and the changes in the assemblages structure usually reflect changes in the environmental conditions (Danovaro et al., 2008; Patrício et al., 2012), making them an effective tool to assess natural and anthropogenic disturbance (Alves et al., 2013, 2015; Fonseca et al., 2011; Materatski et al., 2015, 2016).

Extracellular enzyme activities (EEA) have been used in several studies as a proxy for microbial activity in soils and sediments (Duarte et al., 2008; Pascaud et al., 2012; Ravit et al., 2003). The extracellular enzyme catalyse rate limiting steps of decomposition and nutrient cycling, ultimately affecting the availability of macronutrients otherwise complex or insoluble, and thus unavailable for the biota. This aspect has brought extracellular enzymes to the spotlight regarding enzymatic activity studies in an ecological perspective (Sinsabaugh, 1994). The use of multiple classes of enzymes is recommended in ecological studies, as no single assay can perform as an adequate surrogate for microbial activity (Sinsabaugh, 1994).

The main aim of this study was to assess the disturbance caused by sediment digging of the seagrass beds, through an experimental fieldwork to investigate the responses of the seagrass plant condition, the sediment microbial activity and temporal distribution of the nematode assemblages during the natural recovery of the habitat after digging. The following null hypotheses H_0 were tested: there were no significant differences between control (no digging) and treatments (digging) plots, throughout the 5 sampling occasions *i*) in the photosynthetic efficiency of the *Z. noltii* plants and in the environmental variables measured (grain size, nutrients and organic matter of the sediments); *ii*) in the Extracellular Enzymatic Activity (EEA) of the sediment microbial communities and *iii*) in the nematode assemblage density, biodiversity and trophic composition.

2. Material and methods

2.1. Study area

The study was conducted at the north bank of the Mira estuary (Fig. 1), a small mesotidal system with a semidiurnal tidal regime, which together with the Mira River and its surrounding area is included in the protected Natural Park "Sudoeste Alentejano e Costa Vicentina" (Adão et al., 2009). The lower section of the estuary was characterized by the presence of a large and homogenous *Z. noltii* seagrass bed until its collapse during 2008. However, since 2009 a natural recovery process began, and in 2015 it was possible to observe a considerable big area of seagrass beds naturally recovered. The experimental fieldwork was done at north bank of the lower section of the estuary in the intertidal seagrass bed of *Z. noltii* fully recovered from the 2008 collapse (37°43′ N, 8°45′W), near a private property to guarantee that the seagrass beds are reasonably protected from the harvesting activities since the access to the area is restricted.

2.2. Experimental design

In order to assess the effect of the digging activity on the plant condition (analysed through the photosynthetic efficiency, α), on the nematode assemblages and on the enzymatic activity of microbial communities in the sediments, a total of four experimental plots were randomly selected from 19 plots created in the map of the seagrass bed area chosen for the experimental fieldwork and posteriorly demarcated *in situ*. The four plots were randomly selected by a uniform probability function on the interval {1, 2, 3,..., 19}. Each plot (4 m width × 20 m length) was divided in 16 subplots, with individual areas of 0.79 m^2, distanced 1 m apart from each other, with a buffer area of 10 cm between them and 2 m between subplot rows to preserve the subplots during the sampling procedure.

To simulate the invertebrate harvesting, two plots were subjected to the disturbance created by a turnover of the sediment ("Digging", plot D_1 and plot D_{19}; Fig. 1) and two were set as control plots ("Control", plot C_{11} and plot C_{18}; Fig. 1). The turnover of the sediment occurred one single time (T_0) and was performed with a rake in the first centimetres of the sediment surface by a professional bivalve harvester. The sediment and the *Z. noltti* plants of the treatment plots were left "*in situ*" after digging.

The sampling took place during low tide, in five different occasions: T_0–before digging (May 2015); T_1–14 days after digging (May); T_2–45 days after digging (June); T_3–75 days after digging (July); and T_4–175 days after digging (October). At each sampling occasion, 3 subplots were randomly and unrepeatably selected and sampled the sediment and the *Z. noltii* plants, for biological data as well as grain size analysis, organic matter, interstitial pore water (for salinity and nutrients) analysis and enzymatic activity analysis.

2.3. Sampling and samples treatment

2.3.1. Environmental data

Salinity, pH and Eh (mV) of the sediment interstitial water were measured *in situ* using a VWR pHenomenal® MU600H with pHenomenal® 111 electrode and pHenomenal® OXY 11 probe. Sediment samples for extraction of pore water for N and P (μmol L^{-1}) nutrients analysis were collected with a sediment core (10 cm deep, 5 cm inner diameter); at each sampling occasion, a total of 5 replicates were taken from the 3 randomly selected subplots. Ammonium (NH_4^+) determination was based on the formation of the Indophenol Blue (Koroleff, 1983 *in* Grasshoff and Johannsen, 1972) and nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) concentrations were determined by an adaptation of the Koroleff's protocol (Koroleff, 2007). Relative humidity of the sediment was calculated measuring the fresh weight of the sediment and its weight after dried in an oven at 60 °C until its complete stabilization. Total organic matter was measured following the *Loss on Ignition* (LOI) method (Heiri et al., 2001). Three additional sediment cores (5 cm inner diameter, 10 cm deep) were collected and frozen until further laboratorial analysis of the particle size. All samples were analysed using a Coulter Laser Light Scatter 230 and the following size categories of sediment were determined: clay (<0.004 mm), silt (0.004–0.063 mm), sand (0.063–2 mm) and gravel (>2 mm). The relative content of the different grain size fractions was expressed as a percentage of the total sample weight. Due to the low variability of the sediment

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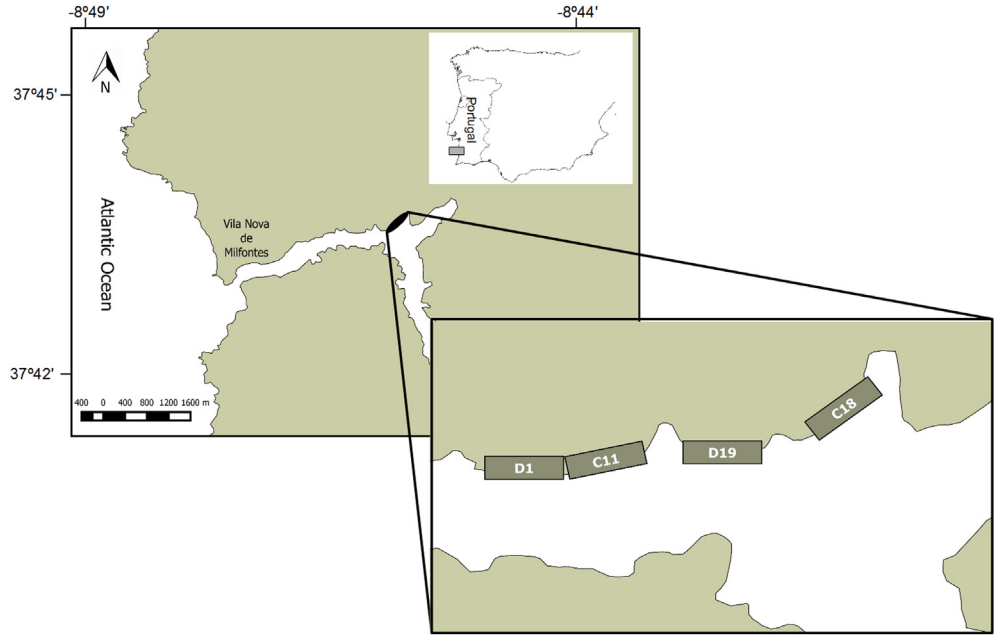

Fig. 1. Mira estuary (Portugal): indication of sampling site and detailed localization of control (C_{11}, C_{18}) and digging (D_1, D_{19}) plots.

composition in a short period of time and the absence of events promoting changes in the Mira estuary hydrodynamics, and after no significant differences of the sediment composition between sampling occasions T1 and T3 were detected, no granulometric measurements were made for the T_2 samples, and the same values obtained at T_1 were used. The photosynthetic efficiency (α) of *Z. noltii* beds was measured *in situ* using a pulse-amplitude modulation (PAM) fluorimeter in light-adapted plants and in plants kept in the dark for 15 min. The photosynthetic apparatus are known to be sensitive to different environmental stress conditions. Therefore, the photochemical efficiency can be used as an indicator to measure the degree of photochemical stress to which the plants are exposed (Duarte et al., 2016).

2.3.2. Microbial community enzymatic activities

For the enzymatic activity determination, sediment cores (10 cm deep, 5 cm diameter) were collected, at each sampling occasion, a total of 5 replicates were taken from the 3 randomly selected subplots. Enzymatic activities of dehydrogenase (intracellular enzyme) and four extracellular enzymes, two oxidoreductases (phenol oxidase and peroxidase), and two hydrolases (β-glucosidase and β-*N*-acetylglucosaminidase (chitobiase)) were determined in the sediment samples.

Dehydrogenase activity was determined according to the TTC method adapted from Thalmann (1968). Approximately 5 g of freshly collected sediment samples were incubated with 2,3,5-triphenyltetrazolium chloride (TTC, 5 ml), in tris-HCl buffer (100 mM). During the incubation (24 h at 30 °C), the reduction of the water-soluble, colorless TTC to the water-insoluble, red 2, 3,5-triphenyltetrazolium formazan (TTF) occurs. TTF was extracted with acetone (40 ml), and after 2 h in the dark the absorbance of the supernatant was read at 546 nm on a TECAN Absorbance Microplate Reader (SPECTRA Rainbow).

For the determination of the extracellular enzymatic activities, the methods in Ravit et al. (2003) were used, with modification of the incubation temperature and without dilution of the supernatant (Reboreda and Caçador, 2008). Sediment slurry was prepared by adding 60 ml of sodium acetate buffer (5 mM) to approximately 5 g of sediment. Different substrates (2 ml) were used depending on the targeted enzyme: *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase (1 h incubation), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide for β-*N*-acetylglucosaminidase (2 h incubation), both releasing *p*-nitrophenol (*p*NP) when hydrolyzed; L-DOPA (L-3,4-dihydroxyphenylalanine) (5 mM) for phenol oxidase (1 h incubation) and L-DOPA (5 mM) + 0.1 ml 0.3% H_2O_2 for peroxidase (1 h incubation). Samples were incubated at 30 °C during the time specified for each enzyme. 1 N NaOH (0.2 ml) was added immediately after incubation to stop the reaction of the *p*NP releasing enzymes. Enzyme and sediment controls were run simultaneously, by subtracting the sediment samples to the former and the respective substrates to the latter. The absorbance of the supernatant was read on a TECAN Absorbance Microplate Reader (SPECTRA Rainbow) at 410 nm for *p*NP enzymes and at 460 nm for L-DOPA enzymes. To calculate the absorbance of peroxidase alone, the absorbance of phenol oxidase was subtracted to the absorbance determined for peroxidase total results.

2.3.3. Biological data

Nematode samples were collected by forcing a hand core (3 cm deep, 3.6 cm inner diameter) at each sampling occasion in each sampling plots (3 replicates). The respective samples were preserved in a 4% buffered formalin solution.

The fixed samples were rinsed using two sieves with different mesh. Each sample was first rinsed on a 1000 μm mesh sieve and then on a 38 μm mesh sieve. The fraction retained was well washed and centrifuged three times using the colloidal silica polymer LUDOX HS-40 (specific gravity 1.18 g cm^{-3}). The supernatant of each centrifugation cycle was collected, abundantly washed on the 38 μm mesh and stored in a 4% formalin solution. After extraction, all nematodes were counted using a stereomicroscope Olympus DP70 (40× magnification) and a counting dish. From each replicate, a random set of approximately 120 nematodes was picked and transferred through a graded series of glycerol-ethanol solutions, stored in anhydrous glycerol and mounted on slides (Heip et al., 1985; Vincx, 1996).

Nematodes were identified to genus level (Olympus BX50 light microscope and cell software D Olympus, Japan). Identification was made using pictorial keys (Platt & Warwick, 1983; Platt & Warwick, 1988; Warwick et al., 1998) and the online identification keys and literature from the Nemys database (Guilini et al., 2016).

2.4. Data analysis

The analysis performed (univariate and multivariate) aimed to detect temporal (sampling occasions, T_0, T_1, T_2, T_3, T_4) and between treatment (digging, D_1, D_{19} and control, C_{11}, C_{18}) changes in the nematode

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assemblages, enzymatic activity and seagrass bed recovery after digging. The statistical analysis of the sediment particle size were calculated using the AnalySize software (Paterson and Heslop, 2015), and the analysis of the extracellular enzymatic activity was performed using the Statistica v.13 software (Dell Inc., 2015).The statistical analysis of the biological and environmental data was performed using the PRIMER v6 software package (Clarke and Gorley, 2006) with the PERMANOVA add-on package (Anderson et al., 2008).

2.4.1. Environmental data

Environmental data was analysed through a Principal Component Analysis (PCA). The analysis was applied to a data matrix based on the average of the replicates analysed at each sampling occasion and collected in the 3 subplots, in order to explore patterns in multidimensional data (Jolliffe, 2002). Data were checked for uniform distribution and, when necessary, a log (X + 1) transformation was performed (water content, ammonium, nitrate, nitrite and phosphate) and data were normalized (Clarke and Gorley, 2006). The redundant variables were removed from the analysis and variables retained in the model act as proxy for the ones that were eliminated (pH, water content, organic matter, salinity, ammonium, nitrate, nitrite, phosphate, clay, mean grain size).

2.4.2. Extracellular enzymatic activity (EEA)

To compare each enzymatic activity across sampling occasion, a nested design analysis of variance (ANOVA) was carried out following a three factor design: "Time": T_0, T_1, T_2, T_3, T_4 (5 levels, fixed), "Treatment": Control and Digging (2 levels, fixed) and "Plot (treatment)": C_{11}, C_{18}, D_1 and D_{19} (4 levels, random). Upon finding significant differences between groups ($\alpha = 0.05$), multiple comparisons were tested using the Bonferroni correction. Spearman correlation analysis was performed to assess the relationship between total organic matter content and enzymatic activities.

2.4.3. Nematode assemblage

Nematode data from each control and digging plot and each sampling occasion were analysed in order to calculate total nematode density (individuals 10 cm^{-2}), genera composition, trophic composition and ecological diversity indexes: Margalef's richness index (d) (Margalef, 1958) and Shannon-Wiener diversity index (H') (Shannon and Weaver, 1963). Indicators based on ecological strategies were also calculated: Index of Trophic Diversity (*ITD*) (Heip et al., 1985) and Maturity Index (*MI*) (Bongers, 1990; Bongers et al., 1991).

To assess the trophic composition of the assemblages, feeding groups based on mouth morphology were assigned to every nematode genus (Wieser, 1953), and the Index of Trophic Diversity (*ITD*) was calculated (Heip et al., 1985). Its reciprocal index (ITD^{-1}) is presented so that the higher value obtained by the index corresponds to the higher trophic diversity. The Maturity Index (*MI*) was used as a life strategy measure, in which a value on a colonizer-persister scale (c–p scale) from 1 (colonizers) to 4 (persisters) was assigned to each genus.

A three-way permutational analysis of variance (PERMANOVA) was applied to the assemblage descriptors (genera composition, number of genera, trophic composition and d, H', ITD^{-1} and *MI* indexes) to test the null hypothesis that the nematode assemblages density, diversity and trophic composition does not significantly change between digging and control plots ("Treatment") and among sampling occasions ("Time"). The PERMANOVA analysis were carried out following a three factor design: "Time": T_0, T_1, T_2, T_3, T_4 (5 levels, fixed), "Treatment": Control and Digging (2 levels, fixed) and "Plot (treatment)": C_{11}, C_{18}, D_1 and D_{19} (4 levels, random), and were conducted on a Bray-Curtis similarity matrix (Clarke and Green, 1988) for genera composition and trophic composition and on Euclidean distance similarity matrices for the univariate data (number of genera, Margalef index, Shannon-Wiener index, ITD^{-1} and *MI*). When the number of permutations was lower than 150, the Monte Carlo permutation p(MC) was used. *A posteriori* pairwise comparisons were performed whenever significant interactions between factors were detected ($p < 0.05$). The similarity in assemblages composition in "Time", "Treatment" and "Plot (treatment)" were plotted by Principal Coordinates Analysis (PCO) using the Bray-Curtis similarity measure. The relative contribution of each genus to the dissimilarities between control and digging plots was calculated using the two way-crossed similarity percentage analysis SIMPER (cut-off percentage 100%).

To analyse and model the relationship between multivariate assemblage structure and environmental variables the DistLM (Distance Based Linear Model) was computed. Environmental variables were checked for high correlations to avoid the collinearity effects, therefore the highly correlated variables were excluded from the analysis. The analysis was performed after checking for uniform distribution and transformation (log (X + 1)) of the variables water content, ammonium, nitrate, nitrite and phosphate. After normalization, DistLM procedure was conducted using the Stepwise selection procedure and the AICc selection criterion (Anderson et al., 2008), whereby they were added to the model the variables that most contributed for differences between "Treatment" and "Time" of the nematode assemblages (organic matter, nitrate, mean grain size). The dbRDA (distance-based redundancy analysis) plot was computed to illustrate the DisTLM model.

3. Results

3.1. Environmental data

The environment variables measured in the sediment and interstitial water in each plot and sampling occasion revealed to be very similar both between treatments and throughout the experiment (Table 1). The majority of the sediment samples were characterized by higher percentage of silt, followed by sand and clay, although in some samples sand was the more abundant granulometric class. The amount of organic matter observed was typical of seagrass bed sediments (±8%). The PCA (PCA1 = 30.7% and PCA2 = 21.4%) ordination of the environmental variables also showed the absence of an evident separation between digging and control plots (Fig. 2). However it is possible to separate samples collected in T_0 and T_1 from those collected in T_2, T_3 and T_4, mainly due the higher values of water content of the sediment, organic matter and nitrite in May 2015 (T_0, T_1), against similar values obtained of the ammonium and phosphate, salinity and clay in June, July and October 2015 (T_2, T_3, T_4) (Fig. 2).

3.2. Zostera noltii seagrass beds

The control and digging plots registered similar photosynthetic efficiency (α) of the *Z. noltii* plants throughout the several sampling occasions. The digging plots showed slightly higher value of α, although an exception is observed in plot D_{19} during T_2 where a low α is observed (Fig. 3).

3.3. Extracellular enzymatic activity

The extracellular enzymatic activity presented a clearly temporal pattern, although no significant differences were obtained between digging and control plots ($p < 0.05$). Significant differences were obtained throughout the sampling occasions for the hydrolases (β-glucosidase and chitobiase) and oxiredutases (oxidade and phenol oxidase) ($p < 0.05$). Significant differences were detected for phenol oxidase in the digging plots. Dehydrogenase activity (DHA) showed significant differences between the digging plot D_{19} and control plot C_{18} (Fig. 4; see Supplementary material).

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Table 1
Environmental variables measured *in situ* in control (C_{11} and C_{18}) and digging (D_1 and D_{19}) plots in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

	Control									
	Plot 11					Plot 18				
Environmental variables	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
pH	7.1	7.5	7.6	7.5	7.6	6.9	7.6	7.6	7.2	7.6
Eh mV	219.7	200.1	193.6	195.5	201.0	219.1	188.4	191.6	209.5	201.8
OM %	10.1	8.8	8.4	8.3	5.9	9.0	8.0	7.7	7.8	4.5
WC %	54.1	50.9	47.3	50.5	50.6	51.9	47.4	46.7	50.6	47.1
Sal	34.0	35.0	35.3	37.7	38.0	39.0	33.7	36.7	37.0	37.7
DIN µmol L^{-1}	113.4	69.6	82.0	220.0	256.4	64.2	64.1	100.4	188.3	226.0
NH_4^+ µmol N L^{-1}	113.1	68.9	81.9	219.3	256.4	64.2	63.8	100.4	188.2	226.0
NO_3^- µmol N L^{-1}	0.0002	0.0002	0.0002	0.0002	0.0000	0.0002	0.0002	0.0002	0.0002	0.0000
NO_2-µmol N L^{-1}	0.319	0.756	0.109	0.662	0.003	0.000	0.321	0.000	0.126	0.000
PO_4^{3-} µmol/L^{-1}	3.9	3.0	4.4	16.9	7.2	1.5	7.3	9.1	4.4	4.7
Clay %	16.4	14.0	14.0	16.3	14.3	15.4	15.2	15.2	18.7	21.5
Silt %	43.9	43.4	43.4	47.7	45.3	44.4	46.5	46.5	47.7	53.2
Sand %	39.7	42.6	42.6	36.0	40.4	40.1	38.2	38.2	33.6	25.4
Gravel %	0	0	0	0	0	0	0	0	0	0
Mean grain size µm	103.5	126.0	126.0	116.0	139.1	110.8	97.7	97.7	89.3	105.9
Environmental variables	Digging									
	Plot 1					Plot 19				
	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
pH	7.3	7.7	7.6	7.5	7.5	7.4	7.3	7.6	7.6	7.5
Eh mV	205.2	186.6	195.6	190.5	203.8	211.3	203.6	195.3	183.5	204.6
OM %	9.7	9.2	8.2	8.4	4.8	9.4	8.9	8.4	9.4	6.4
WC %	53.4	57.4	49.3	47.2	47.2	52.6	63.3	50.2	50.8	51.8
Sal	34.7	37.3	36.7	38.0	35.8	36.3	34.3	36.7	40.0	37.0
DIN µmol L^{-1}	122.4	128.4	262.1	168.5	176.5	150.9	337.0	304.2	137.7	137.9
NH_4^+ µmol N L^{-1}	122.2	127.8	262.1	168.4	176.5	150.7	336.7	304.1	137.7	118.3
NO_3^- µmol N L^{-1}	0.0002	0.0002	0.0002	0.0002	0.0000	0.0002	0.0002	0.0002	0.0002	0.0000
NO_2^- µmol N L^{-1}	0.171	0.577	0.021	0.084	0.020	0.162	0.313	0.021	0.010	0.247
PO_4^{3-} µmol/L^{-1}	1.4	1.8	12.1	5.1	2.7	4.8	13.5	16.2	3.8	4.4
Clay %	15.7	14.6	14.6	15.9	16.5	13.2	16.8	16.8	17.5	16.5
Silt %	42.6	40.5	40.5	46.2	44.4	41.9	45.2	45.2	47.1	44.3
Sand %	41.7	44.8	44.8	37.9	39.1	45.0	38.0	38.0	35.4	39.2
Gravel %	0	0	0	0	0	0	0	0	0	0
Mean grain size µm	122.4	131.6	131.6	120.3	143.7	134.2	99.6	99.6	98.4	124.4

pH, potential of hydrogen; Eh, oxidation-reduction potential; OM, organic matter content; WC, water content; Sal, Salinity; DIN – dissolved inorganic nitrogen; NH_4^+, ammonium; NO_3^-, nitrate; NO_2^-, nitrite; PO_4^{3-}, phosphate; clay < 0.004 mm; Silt 0.004–0.0625 mm; Sand 0.0625–2 mm; gravel >2 mm; mean grain size.

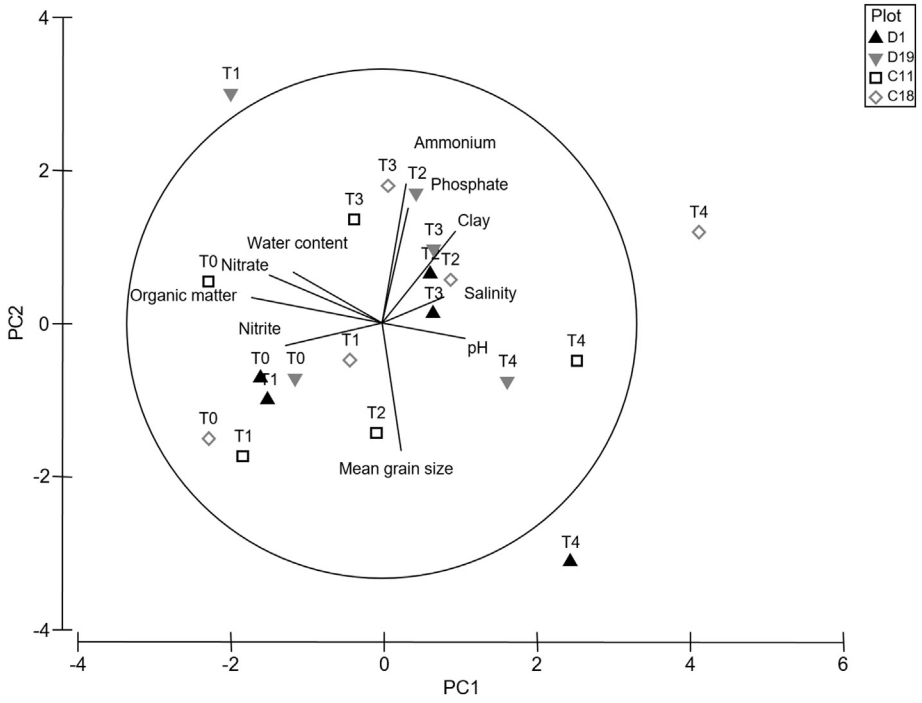

Fig. 2. Principal Component Analysis (PCA) plot based on the environmental variables measured in five sampling occasions ("Time": 5 levels, fixed), under control and digging treatments ("Treatment: 2 levels, fixed) performed in multiple plots ("Plot (treatment)": 4 levels, random). Vectors length corresponds to the correlation values. PCA1 = 30.7% and PCA2 = 21.4%.

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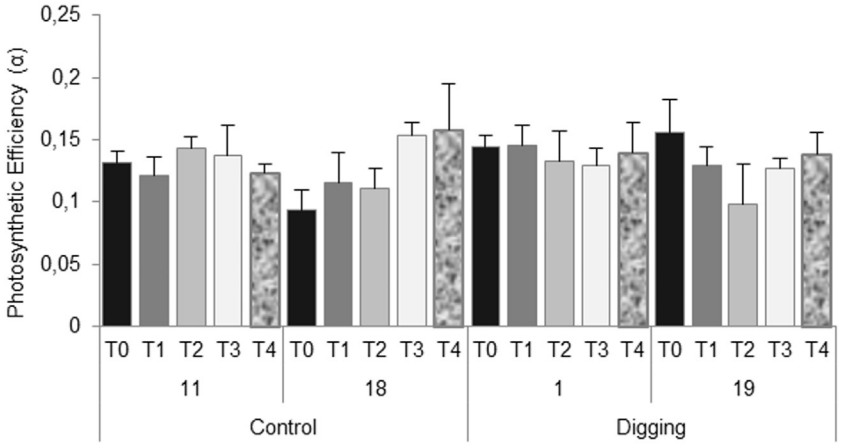

Fig. 3. Mean photosynthetic efficiency ± standard error (SE) of *Z. noltii* seagrass beds in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

3.4. Nematode assemblages-density

Overall, the nematode density ranged from 599 to 8486 ind. 10 cm^{-2}. In control plots the mean density was 3603 ± 431 ind. 10 cm^{-2}, with minimum nematode densities found in the plot C_{11} (1580 ± 357 ind. 10 cm^{-2}) at T_4 and maximum densities in the plot C_{18} (5264 ± 553 ind. 10 cm^{-2}) at T_1 (Table 2; see supplementary material). The digging plots presented the mean density of 4461 ± 583 ind. 10 cm^{-2}, with minimum values in plot D_{19} (2280 ± 559 ind. 10 cm^{-2}), at T_4 and maximum values in plot D_1 (6540 ± 1546 ind. 10 cm^{-2}), at T_3 (Table 2; see Supplementary material). The assemblage-based PCO ordination reflected the low variability in the nematode density that did not allow a distinction between control and treatment plots (Fig. 5).

PERMANOVA analysis for the nematode density revealed significant differences for factors "Time" and "Plot (Treatment)" and their interactions ($p < 0.05$, for all) (Table 3). Individual pairwise comparisons on interaction factor "Time × Plot (Treatment)" revealed that control plots maintained similar densities throughout the experiment while their density between digging plots was significantly different at T_0 and T_4. Also, the assemblage density among plot D_1 was significantly lower at T_4 than at the remaining sampling occasions ($p < 0.05$, for all).

3.5. Nematode assemblage composition-structural diversity

Overall, 67 nematode genera from 21 families and 3 orders were identified. The order composition for both control and treatment plots presented similar percentages with most of the genera belonging to Monhysterida (51.5%), followed by Chromadorida (45.1%) and Enoplida (3.5%). The control plots presented a total of 59 genera with 91.3% of the assemblage being composed by Linhomoidae (37.3%) that had the higher representative percentage, followed by Comesomatidae (23.7%), Chromadoridae (13.4%), Axonolaimidae (6.8%), Xyalidae (6.2%) and Desmodoridae (3.8%). In the treatment plots, 55 genera were registered, and as at the control plots, Linhomoidae (38.3%) represented the highest percentage of the assemblage, followed by Comesomatidae (25.6%), Chromadoridae (13.7%) and Axonolaimidae (6.2%), Xyalidae (6.0%) and Desmodoridae (2.6%). Together they comprise 92.4% of the assemblage.

More, at the control and treatment plots, *Terschellingia, Paracomesoma, Ptycholaimellus, Linhomoeus, Sabatieria, Daptonema* and *Odontophora* were the most abundant genera, contributing for approximately 80% of the nematode assemblages. The SIMPER analysis provided information about the genera contribution to the similarity and dissimilarity between the treatment, the results obtained revealed

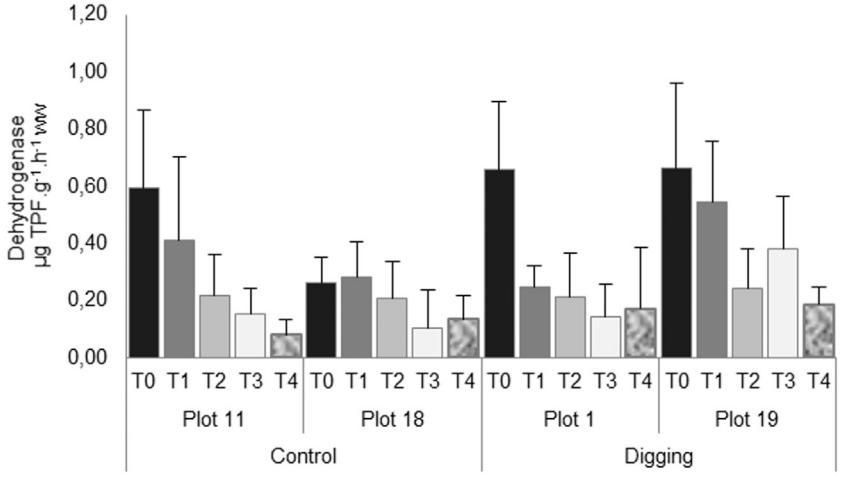

Fig. 4. Dehydrogenase activity (DHA) (µg TPF.g^{-1}.h^{-1} ww) ± standard error (SE) of *Z. noltii* seagrass beds sediment in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

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Table 2
Mean density ± standard error (SE) of nematode genera (number of individuals per 10 cm^{-2}) in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4). Trophic group (TG) and *c–p* value of each genera included. Only the most abundant genera are included in this table.

Genera	TG	*c–p* value	Control									
			Plot 11					Plot 18				
			T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
Terschellingia	1A	3	695 ± 119	301 ± 81	366 ± 83	291 ± 78	298 ± 112	303 ± 107	2076 ± 244	528 ± 170	1998 ± 1385	514 ± 218
Paracomesoma	1B	2	326 ± 69	440 ± 245	688 ± 65	598 ± 200	522 ± 152	225 ± 99	449 ± 50	474 ± 154	675 ± 244	320 ± 149
Linhomoeus	2A	2	247 ± 55	310 ± 214	446 ± 294	414 ± 297	167 ± 80	224 ± 69	104 ± 34	445 ± 83	432 ± 308	173 ± 136
Ptycholaimellus	2A	3	236 ± 7	214 ± 127	83 ± 60	33 ± 17	17 ± 10	457 ± 213	916 ± 523	420 ± 159	203 ± 75	98 ± 50
Sabatieria	1B	2	74 ± 35	133 ± 51	51 ± 23	146 ± 61	177 ± 81	358 ± 149	274 ± 86	300 ± 19	313 ± 226	76 ± 43
Daptonema	1B	2	244 ± 18	126 ± 74	198 ± 85	114 ± 36	101 ± 29	290 ± 49	157 ± 83	150 ± 31	137 ± 57	44 ± 6
Odontophora	2A	2	166 ± 53	93 ± 36	70 ± 47	114 ± 46	81 ± 21	237 ± 154	144 ± 36	176 ± 78	170 ± 69	75 ± 34
Metachromadora	1B	2	262 ± 192	19 ± 10	36 ± 24	0	0	131 ± 52	73 ± 29	106 ± 56	217 ± 149	55 ± 29
Axonolaimus	1B	2	23 ± 12	50 ± 34	11 ± 11	0	0	171 ± 81	179 ± 100	128 ± 63	40 ± 12	14 ± 1
Atrochromadora	2A	4	0	0	0	0	0	148 ± 82	206 ± 88	37 ± 23	96 ± 78	22 ± 15
Sphaerolaimus	2A	2	14 ± 14	23 ± 9	15 ± 8	33 ± 17	17 ± 10	9 ± 9	103 ± 54	59 ± 37	14 ± 14	8 ± 8
Metalinhomoeus	1B	2	14 ± 14	13 ± 13	0	7 ± 7	6 ± 6	147 ± 147	106 ± 106	0	33 ± 18	9 ± 5
Anoplostoma	2B	3	7 ± 7	2 ± 2	0	0	0	30 ± 24	196 ± 67	10 ± 10	104 ± 104	21 ± 21
Other genera			571 ± 2	150 ± 1	384 ± 2	213 ± 1	190 ± 1	397 ± 2	370 ± 2	473 ± 3	332 ± 2	151 ± 1
Total			2878 ± 99	1873 ± 205	2349 ± 156	1964 ± 150	1575 ± 76	3127 ± 169	5353 ± 133	3308 ± 50	4766 ± 319	1580 ± 19

Genera	TG	*c–* value	Digging									
			Plot 1					Plot 19				
			T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
Terschellingia	1A	3	1345 ± 94	1376 ± 968	1829 ± 689	2443 ± 1170	366 ± 121	677 ± 135	905 ± 278	742 ± 371	1011 ± 502	614 ± 367
Paracomesoma	1B	2	545 ± 128	320 ± 68	525 ± 178	556 ± 288	1476 ± 315	1082 ± 182	978 ± 229	522 ± 263	615 ± 438	369 ± 190
Linhomoeus	2A	2	498 ± 192	436 ± 161	750 ± 308	306 ± 133	250 ± 171	201 ± 33	596 ± 211	761 ± 606	1151 ± 514	269 ± 38
Ptycholaimellus	2A	3	594 ± 83	371 ± 70	529 ± 304	592 ± 33	8 ± 8	466 ± 192	861 ± 200	719 ± 199	450 ± 107	0
Sabatieria	1B	2	170 ± 65	241 ± 193	668 ± 264	1181 ±	105 ± 6	271 ± 226	563 ± 86	527 ± 185	380 ± 218	150 ± 125
Daptonema	1B	2	220 ± 67	76 ± 38	327 ± 116	314 ± 111	15 ± 8	318 ± 61	445 ± 135	271 ± 184	319 ± 146	159 ± 63
Odontophora	2A	2	289 ± 131	193 ± 55	378 ± 143	183 ± 55	31 ± 31	280 ± 85	151 ± 68	111 ± 23	291 ± 182	128 ± 50
Metachromadora	1B	2	14 ± 14	127 ± 44	43 ± 23	206 ± 85	0	75 ± 7	110 ± 33	76 ± 50	170 ± 66	104 ± 40
Axonolaimus	1B	2	14 ± 14	0	46 ± 24	46 ± 23	30 ± 9	162 ± 90	284 ± 157	103 ± 65	0	6 ± 6
Atrochromadora	2A	4	0	0	0	0	0	347 ± 179	17 ± 17	89 ± 72	140 ± 56	0
Sphaerolaimus	2A	2	14 ± 14	48 ± 34	27 ± 27	81 ± 6	45 ± 25	10 ± 10	25 ± 15	50 ± 34	106 ± 40	0
Metalinhomoeus	1B	2	0	19 ± 19	46 ± 24	50 ± 25	0	88 ± 88	0	7 ± 7	156 ± 146	26 ± 4
Anoplostoma	2B	3	49 ± 9	57 ± 57	27 ± 16	75 ± 43	0	13 ± 13	42 ± 42	7 ± 7	45 ± 25	61 ± 61
Other genera			394 ± 2	261 ± 2	531 ± 3	505 ± 4	93 ± 1	639 ± 4	755 ± 6	471 ± 3	317 ± 2	389 ± 3
Total			4146 ± 94	3526 ± 266	5726 ± 361	6540 ± 327	2420 ± 68	4628 ± 86	5733 ± 300	4457 ± 277	5150 ± 294	2275 ± 119

that nematode genera that contributed the most for the similarity within the control and treatment plots were also great contributors for the biggest dissimilarities between them (*Terschellingia, Paracomesoma, Limonhoeus, Ptycholaimellus* and *Sabatieria*). The nematode assemblages' structural diversity revealed significant differences for the factors "Time" and "Plot (treatment)" (Table 3). Individual pairwise tests revealed significant differences for the number of genera between T_0 and T_4 and within the treatment plots D_1 and D_{19} ($p < 0.05$).

Genera diversity based on Shannon-Wiener index (H') registered the highest value at T_0 ($H' = 2.6$) in plot C_{18} and the lowest value ($H' = 1.2$) at T_4 in plot D_{19} (Fig. 6). PERMANOVA analysis for genera diversity based on Shannon-Wiener index (H') revealed significantly differences between "Time × Plot (Treatment)" ($p < 0.05$) (Table 3). Individual pairwise comparisons between interaction factors revealed that the diversity was significantly different at T_4 within treatment plots. Moreover, plot D_1 showed significantly lower diversity at T_4 that in the remaining sampling occasions ($p < 0.05$, for all).

Nematode richness based on Margalef Index (d) registered the highest value in plot C_{11} ($d = 2.4$) at T_0 and the lowest value ($d = 1.02$) in plot D_1 at T_4 (Fig. 6). PERMANOVA analysis for Margalef richness revealed significantly differences between "Time × Plot (Treatment)" ($p < 0.05$) (Table 3). Individual pairwise comparisons for the interaction "Time × Plot (Treatment)" showed that Margalef richness was lower in plot D_1 than in D_{19} at the end of the experiment (T_4) and that, in general, Margalef richness in plot D_1 was significantly lower in T_4 than in almost of the remaining occasions ($p < 0.05$).

3.6. Nematode assemblages-trophic composition and functional diversity

In general, the dominance of trophic groups was similar for both control and treatment plots (Fig. 7). The control plots were characterized mainly by non-selective deposit feeders (1B: 38.7 ± 3.7%) and epigrowth feeders (2A: 30.4 ± 4.4%) that encompassed approximately 69% of the nematode assemblage, with selective deposit feeders (1A: 28.5 ± 0.3%) and omnivores/predators (2B: 2.3 ± 0.3%) representing only 31% of the nematode assemblage. Similar results were obtained in the digging plots, with 71% of the assemblage being characterized by non-selective deposit feeders (1B: 38.2 ± 3%) and epigrowth feeders (2A: 32.7 ± 5.8%) and the remaining 29% of the assemblage comprising selective deposit feeders (1A: 27.1 ± 4.4%) and omnivores/predators (2B: 2.0 ± 0.6%) (Fig. 7). PERMANOVA analysis for the nematode trophic composition revealed significantly differences between "Plot (treatment)" ($p < 0.05$) (Table 3). Individual pairwise comparisons showed that only the trophic composition within control plots was significantly different ($p < 0.05$).

The Index of Trophic Diversity ranged from 2.0 ± 0.06 (plot C_{18}, T_3) to 3.1 ± 0.2 (plot C_{11}, T_0) in the control plots, and from 1.6 ± 0.2 (plot D_1, T_4) to 2.7 ± 0.27 (plot D_1, T_3) in treatment plots (Fig. 8). PERMANOVA analysis for the ITD^{-1} indicated no significant differences in the trophic diversity ($p > 0.05$, for all) for the nematode assemblages (Table 3).

Maturity index (MI) vary from 2.3 ± 0.04 (plot C_{11}, T_3) to 2.7 ± 0.004 (plot C_{18}, T_1) in the control plots, and from 2.2 ± 0.06 (plot D_1,

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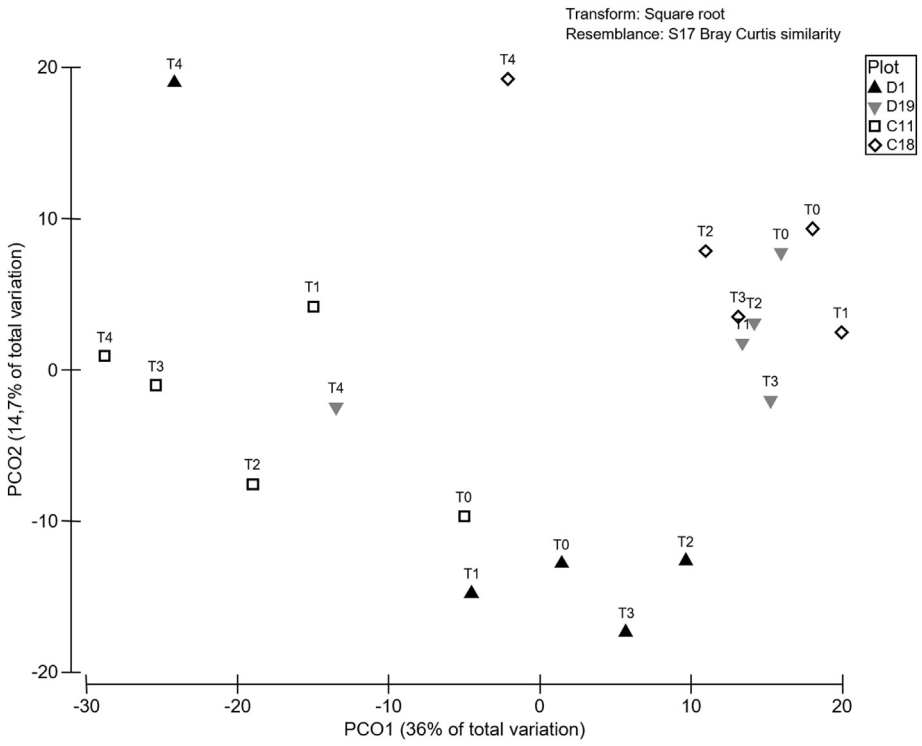

Fig. 5. Principal coordinates analysis (PCO) based on the nematode densities in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion ("Time": T_0, T_1, T_2, T_3, T_4). PCO1 = 36%; PCO2 = 14.7%.

T_4) to 2.5 ± 0.05 (plot D_1, T_0) in the treatment plots. From the *MI* it was possible to observe that most genera belong to the colonizer category known as 'general opportunists' (*c–p* value 2), in both the control (57%) and digging (58%) plots, followed by the 'intermediate *c–p* group' (*c–p* value 3) that represented approximately 39–40% of the assemblage, in control plots and treatment plots respectively (Fig. 8). PERMANOVA analysis for the *MI* revealed significant differences ($p < 0.05$) between "Plot (treatment)" (Table 3), and individual pairwise comparison showed that those differences were only within control plots ($p < 0.05$).

3.7. Environmental data vs. nematode assemblages

The marginal (individual variables) tests on the DistLM analysis indicated that organic matter, nitrate and mean grain size explain a significant amount of the variation in nematode genera composition ($p < 0.05$). The sequential tests using the AICc selection procedure indicate that these environmental variables constitute the best explanatory model for the nematode assemblage patterns (dbRDA1 = 65.7% and dbRDA2 = 23.6%). These results imply that organic matter and nitrate explain a large part of the variability observed in the nematode assemblages in both control and digging plots at T_0 and T_2 (Fig. 9).

4. Discussion

The seagrass beds may be impacted by anthropogenic physical disturbances such as the disturbances associated with harvesting operations that were proven to adversely affect them (Orth et al., 1998) and to reduce the mudflats biodiversity (Brown and Wilson, 1997). The resulting reductions in seagrass biomass may take years to recover to the existing levels previous to disturbance (Boese, 2002). At the Mira estuary the seagrass beds of *Z. noltii* are in a natural recovery process after a major collapse during the year of 2008 and there are some beds entirely recovered while other beds seem to be definitively lost; meanwhile the mechanical activity of harvesting seems to be increasing which could compromise the natural recovery process in progress.

The digging activity for bivalve collecting involves the physical disturbance of the sediments, and depending on its frequency and intensity, such activities may have an impact on the structure and the interactions of the ecosystem (primary production, nutrient cycling and assemblage structure) (Day et al., 2013). The physical disturbance of the sediments is an important driver for both the spatial and temporal density and diversity patterns of the benthic assemblages, and may lead to the displacement of species to adjacent unfavorable habitats or, in extreme cases, to the complete defaunation when there is a total removal of sediment (Ramsay and Kaiser, 1998). Contrariwise, the physical disturbance performed during this field experiment in the intertidal sediment of the seagrass bed of the Mira estuary clearly allowed a rapid habitat recovery and no symptoms of habitat disruption were detected.

The digging event permitted the rapid plant recovery, with the control and digging plots presenting similar values of the photosynthetic efficiency of the *Z. noltii* plants in all the sampling occasions. These results were also observed in Yaquina Bay (USA) in eelgrass meadow, after the recreational clam raking the *Z. marina* recovered rapidly from this type of physical disturbance (Boese, 2002).

The sediment microbial activities were shown to be sensitive to the environmental changes constituting a good indicator of the sediment quality status; the EEA have been used to provide information on the microbial community status (Duarte et al., 2012). The EEA results present seasonal variability between May and October 2015 corresponding to the duration of the field experiment. Like other variables measured in the present work EEA were insensitive to the digging activity. The variability observed in the several enzymes analysed can be explained mainly by the biogeochemical processes of the intertidal estuarine sediments. The EEA are generally indicative of nutrient limitation and nutrient availability regulations, since microbiological communities control the production of such enzymes to acquire limiting nutrients (Bowles et al., 2014). Phenol oxidase and peroxidase EEA were higher than what was observed for glucosidase and chitobiase. The dominance of the oxireductases, which are involved in the breakdown of organic matter, over the hydrolases may indicate the presence of complex organic substrates in the sediment (Reboreda and Caçador, 2008). Sediment

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Table 3
Three-way PERMANOVA test with "Time" (5 levels, fixed), "Treatment" (2 levels, fixed) and "Plot" (2 levels, random and nested in "Treatment") for all variables analysed. Bold values represent significant effects and interactions ($p < 0.05$).

Source of variation	Degree of freedom	Sum of squares	Mean square	Pseudo-F	Unique perms	P(MC)	P(perm)
Nematode density							
Time	4	7901.1	1975.3	1.8063	999	0.014	**0.03**
Treatment	1	2836.3	2836.3	0.68767	3	0.719	0.664
Plot (treatment)	2	8249.1	4124.5	5.0532	999	0.001	**0.001**
Time × treatment	4	3535.1	883.77	0.80818	998	0.757	0.695
Time × plot (treatment)	8	8748.2	1093.5	1.3397	998	0.047	0.026
Residual	40	32,649	816.23				
Total	59	63,919					
Number of genera							
Time	4	1376.3	344.07	3.1154	999	0.072	**0.015**
Treatment	1	669.66	669.66	2.6902	3	0.212	0.317
Plot (treatment)	2	497.85	248.93	4.0002	997	0.02	**0.018**
Time × treatment	4	501.52	125.38	1.1352	999	0.404	0.402
Time × plot (treatment)	8	883.54	110.44	1.7748	998	0.097	0.1
Residual	40	2489.1	62.228				
Total	59	6418					
Shannon-Wiener index							
Time	4	1.3887	0.34716	1.8071	999	0.225	0.178
Treatment	1	0.049001	0.049001	0.61488	3	0.549	0.647
Plot (treatment)	2	0.15938	0.079692	1.1607	998	0.322	0.315
Time × treatment	4	0.44916	0.11229	0.5845	999	0.688	0.74
Time × plot (treatment)	8	1.5369	0.19211	2.798	999	0.013	**0.011**
Residual	40	2.7464	0.068661				
Total	59	6.3295					
Margalef index							
Time	4	1.0716	0.2679	1.2402	999	0.351	0.357
Treatment	1	1.2203	1.2203	9.5585	3	0.09	0.328
Plot (treatment)	2	0.25534	0.12767	1.3357	998	0.266	0.268
Time × treatment	4	0.53925	0.13481	0.62408	999	0.65	0.697
Time × plot (treatment)	8	1.7281	0.21602	2.2601	999	0.057	**0.046**
Residual	40	3.8232	0.09558				
Total	59	8.6379					
Trophic composition							
Time	4	2349.8	587.45	1.737	998	0.14	0.165
Treatment	1	1649.8	1649.8	2.0217	3	0.2022	0.353
Plot (treatment)	2	1632.2	816.08	3.8672	999	0.008	**0.008**
Time × treatment	4	436.89	109.22	0.32294	999	0.97	0.96
Time × plot (treatment)	8	2705.7	338.21	1.6027	997	0.079	0.068
Residual	40	8441.1	211.03				
Total	59	17,215					
Index of Trophic Diversity							
Time	4	1.618	4.05E-01	1.8165	998	0.212	0.188
Treatment	1	1.98E-03	1.98E-03	5.68E-03	3	0.942	0.656
Plot (treatment)	2	0.69655	3.48E-01	2.2019	998	0.1	0.141
Time × treatment	4	0.73352	1.83E-01	0.82352	999	0.538	0.54
Time × plot (treatment)	8	1.7814	2.23E-01	1.4078	999	0.208	0.211
Residual	40	6.327	1.58E-01				
Total	59	11.158					
Maturity index							
Time	4	0.17325	0.043312	1.4382	998	0.311	0.327
Treatment	1	2.17E-04	2.17E-04	0.002346	3	0.971	1
Plot (treatment)	2	0.1853	0.092651	6.0208	998	0.005	**0.004**
Time × treatment	4	0.15765	0.039412	1.3087	999	0.33	0.317
Time × plot (treatment)	8	0.24093	0.030116	1.957	999	0.087	0.084
Residual	40	0.61554	0.015388				
Total	59	1.3729					

organic matter cycling is expected to be higher with higher enzymatic activities, but plant roots may also be contributing to higher peroxidase EEA by releasing peroxidases into the sediments (Vaughan et al., 1994).These may explain the increase in peroxidase EEA, despite the decrease of the organic matter content throughout the sampling occasions. The DHA activity reflects the metabolism (*i.e.*, total oxidative activity) of the microbial community present in the sediments. The gradual decreased observed over time (May to October) may indicate lower metabolic capacity or lower density of the microbial communities in the sampled sediment. The observed salinity and water content variations throughout the sampling occasions may explain the decrease in the potential DHA activity (Brzezińska et al., 1998; Caravaca et al., 2005; Carrasco et al., 2006; Marzadori et al., 1996).

The variability of the nematode assemblages observed in control and digging plots was not a response to the digging activity but to the physicochemical conditions, trophic dynamics and biological factors characteristics of the seagrass bed environments. As a matter of fact, the mean grain size, nitrites, nitrates and organic matter content revealed to be

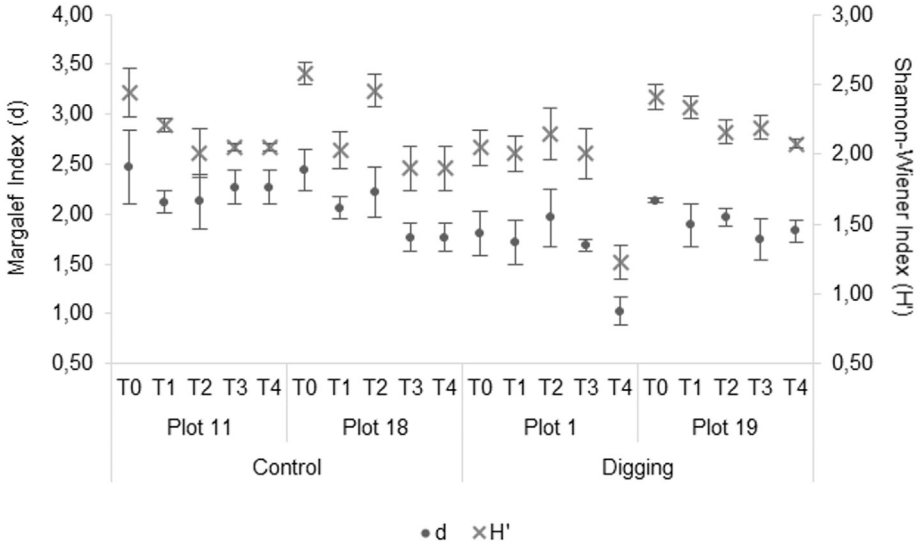

Fig. 6. Mean values ± standard error (SE) for Margalef Index (d) and Shannon-Wiener Index (H') in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

fundamental for structuring the observed distribution of the nematode assemblages. The high density, diversity and low variability of the assemblages are probably the result of high food availability typical of the seagrass beds. The organic carbon input for the nematodes food web in seagrass beds at the Mira estuary was already studied, and various sources were identified, *i.e.* seagrass detritus, epiphytes, microphytobenthos and suspended particulate organic matter (Vafeiadou et al., 2014). The seagrass sediments were populated mainly by non-selective deposit feeders (1B) and epistrate feeders (2A) and as expected the values of ITD^{-1} and *MI* are similar in the control and the digging plots and the high trophic diversity obtained is commonly found in muddy and seagrass substrata (Fonseca et al., 2011; Materatski et al., 2016).

In traditional harvesting, the target fauna are extracted using suction pumps, by digging or by raking that only remove individual species and the sediment surface is left in place, its composition is not affected by the sediment turnover and the raked areas are small, which attenuate potential effects. It is possible to support that sediment physical disturbance and benthic species abundance and diversity will not be necessarily disturbed during harvesting as is supported by the results of this experimental fieldwork. Nematode assemblages showed strongly resilience to single events of mechanical disturbance at small scale, only one tidal cycle is required to recolonize disturbed sediments and after 12 h densities of major groups return to the pre-disturbance levels (Sherman and Coull, 1980). Experimental studies showed that nematodes are able to actively migrate through sediments, penetrate to deeper layers and recolonize the defaunated sediments *via* active lateral interstitial migration (Schratzberger et al., 2000; Schratzberger et al., 2004).

The physical disturbance of the *Z. noltii* seagrass beds caused by traditional harvesting allowed a rapid natural recovery of the habitat, following the turnover of the sediment. The photosynthetic efficiency of the plants, sediment environmental variables Extracellular Enzymatic Activity (EEA) of the sediment and nematode assemblages' abundance and diversity are in accordance the characteristics of the intertidal seagrass bed including the results obtained in previous studies in Mira estuary (Adão, 2004; Materatski et al., 2015, 2016).

5. Conclusion

The field experiment presented in this work demonstrated the clear capacity of the seagrass beds of *Z. noltii* to recover under low intensity

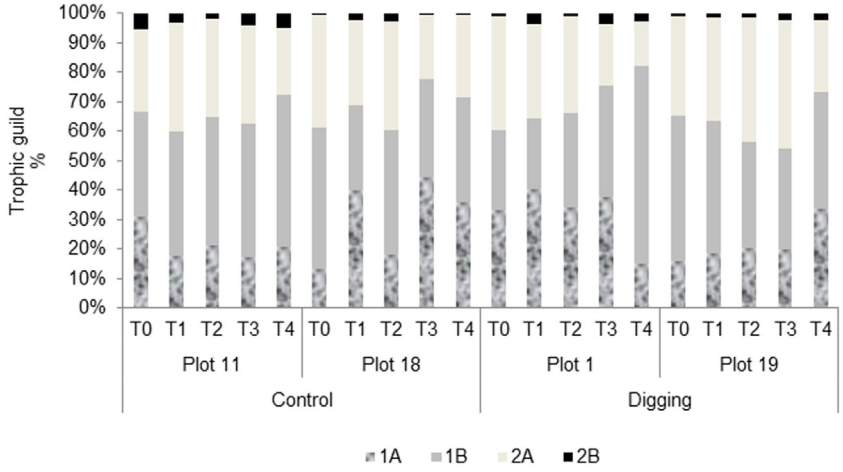

Fig. 7. Trophic guild composition (1A – selective feeders; 2A epigrowth feeder; 1B – non-selective feeders; 2B – omnivores/predators) in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

Please cite this article as: Branco, J., et al., Natural recovery of *Zostera noltii* seagrass beds and benthic nematode assemblage responses to physical disturbance caused by traditional harvesting activities..., J. Exp. Mar. Biol. Ecol. (2017), http://dx.doi.org/10.1016/j.jembe.2017.03.003

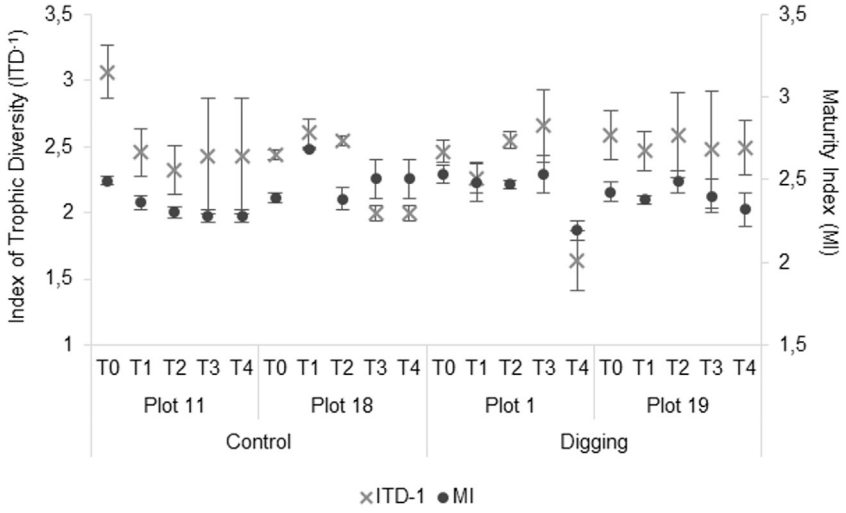

Fig. 8. Mean values ± standard error (SE) of the Index of Trophic Diversity (ITD^{-1}) and Maturity Index (MI) in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plot in each sampling occasion (T_0, T_1, T_2, T_3, T_4).

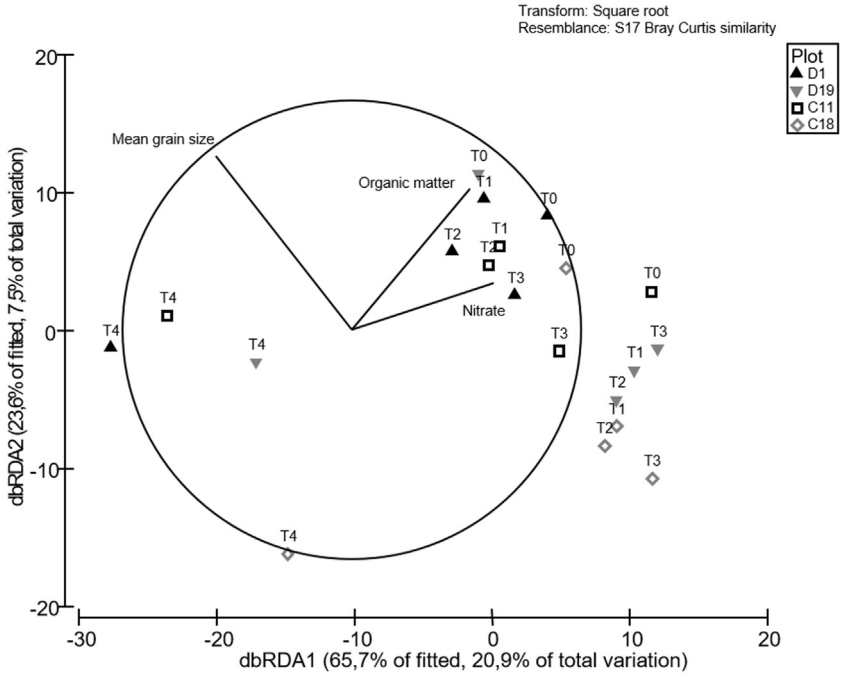

Fig. 9. Distance-based redundancy (dbRDA) plot illustrating the DistLM model (Step-wise procedure; AICc selection criterion) based on the environmental variables that significantly determined nematode genera distribution in control (C_{11}, C_{18}) and digging (D_1, D_{19}) plots in each sampling occasions (T_0, T_1, T_2. T_3, T_4). dbRDA1 = 23.6% fitted; 7.5% total and dbRDA2 = 65.7% fitted; 20.9% total.

physical disturbance associated to harvesting. Nevertheless, the harvesting activity is increasing in Mira estuary and the seagrass bed responses to intense physical disturbance were not evaluated. The results included in the current paper will provide the baseline to assess the habitat responses to intense physical disturbance and it will be essential to ensure that the collective pressure of human activities on the environment is kept within levels compatible with the sustainable use of a diverse marine and estuarine ecosystem.

Acknowledgements

This work was supported by the Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2013 and by the research projects ProMira (PROMAR; 31-03-02-FEP-006) and CoolNematode (FCT; EXPL/MAR-EST/0553/2013).

A special thanks to all that assisted us during field and laboratory work. **[SW]**

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2017.03.003.

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Please cite this article as: Branco, J., et al., Natural recovery of *Zostera noltii* seagrass beds and benthic nematode assemblage responses to physical disturbance caused by traditional harvesting activities..., J. Exp. Mar. Biol. Ecol. (2017), http://dx.doi.org/10.1016/j.jembe.2017.03.003