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A comparative analysis of benthic nematode assemblages from *Zostera noltii* beds before and after a major vegetation collapse

Patrick Materatski [a, *], Anna-Maria Vafeiadou [b], Rui Ribeiro [c], Tom Moens [b], Helena Adão [a]

[a] MARE – Marine and Environmental Sciences Centre, University of Évora, School of Sciences and Technology, c/o NemaLab, Apartado 94, 7002-554 Évora, Portugal
[b] Ghent University, Marine Biology Section, Department of Biology, Krijgslaan 281/S8, 9000 Ghent, Belgium
[c] CFE – Centre for Functional Ecology, University of Coimbra, Department of Life Sciences, Calçada Martim de Freitas, 3000-456, Coimbra, Portugal

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ABSTRACT

Benthic nematodes are widely regarded as very suitable organisms to monitor potential ecological effects of natural and anthropogenic disturbances in aquatic ecosystems. During 2008, the seagrass beds of *Zostera noltii* located in the Mira estuary (SW Portugal) disappeared completely. However, during 2009, slight symptoms of natural recovery were observed, a process which has since evolved intermittently. This study aims to investigate changes in patterns of nematode density, diversity, and trophic composition between two distinct habitat conditions: "before" the collapse of seagrass beds, and during the early recovery "after" the seagrass habitat loss, through the analysis of: i) temporal and spatial distribution patterns of nematode communities, and ii) the most important environmental variables influencing the nematode assemblages. The following hypotheses were tested: i) there would be differences in nematode assemblage density, biodiversity and trophic composition during both ecological conditions, "before" and "after"; and ii) there would be differences in nematode assemblage density, biodiversity and trophic composition at different sampling occasions during both ecological conditions. Nematode density and diversity were significantly different between the two ecological situations. A higher density was recorded before, but a higher diversity was evident after the collapse of *Z. noltii*. In spite of the disturbance caused by the seagrass habitat loss in the Mira estuary, the nematode trophic composition did not significantly differ between the before and after seagrass collapse situations. Despite the significant differences found among sampling occasions, a consistent temporal pattern was not evident. The response of nematode communities following this extreme event exhibited considerable resistance and resilience to the new environmental conditions.

1. Introduction

Benthic nematodes provide valuable information regarding ecosystem health (Sheppard, 2006). Sediment structure, chemistry, disturbance and availability of food, such as bacteria and microphytobenthos, are closely linked to nematode assemblage composition and distribution patterns (Giere, 1993; Heip et al., 1985; Moens et al., 2005), through the changes in density, diversity, structure and functioning (Danovaro et al., 2008; Norling et al., 2007; Patrício et al., 2012). Furthermore, several studies have highlighted the importance of the link between nematode diversity and ecosystem functioning, which may be important in the assessment of estuarine and marine biological integrity (Coull and Chandler, 1992; Danovaro et al., 2008; Fonseca et al., 2011; Moreno et al., 2008; Schratzberger et al., 2004; Steyaert et al., 2007).

Seagrass beds provide habitat for ecological communities and enhance biodiversity through their facilitative effects on associated species (Ellison et al., 2005), acting as ecosystem engineers by structuring pelagic and benthic assemblages (Bos et al., 2007). Seagrass beds are important in primary production, nutrient cycling, sediment and nutrient trapping, sediment stabilization, and their structural complexity is critical for the animals which live in them (Boström and Bonsdorff, 1997; Orth et al., 2006). Several studies that analysed the meiobenthic communities associated with seagrass beds have concluded that meiofauna in vegetated

* Corresponding author. MARE – Marine and Environmental Sciences, School of Sciences and Technology, University of Évora, c/o NemaLab, Apartado 94, 7002-554 Évora, Portugal.
E-mail address: pmateratski@uevora.pt (P. Materatski).

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sediments is more abundant and diverse than in adjacent bare sediments (Castel et al., 1989; Fisher and Sheaves, 2003; Guerrini et al., 1998; Ndaro and Olafsson, 1999).

There have been numerous reports of seagrass decline around the world indicating that seagrass habitats are undergoing a global crisis threatening associated organisms and ecosystem functions (Hughes et al., 2009; Valle et al., 2014). Although sometimes caused by natural disturbances, most declines are attributed to anthropogenic disturbances (Short and Wyllie-Echeverria, 1996). Along the Portuguese coast, seagrass populations are also facing unprecedented declines in distribution, matching the general trends described for seagrasses worldwide (Cunha et al., 2013). The *Zostera noltii* (Hornem.) seagrass beds in the Mira estuary have been a prominent habitat since the 90s (Almeida, 1994; Costa, 2004; Costa et al., 1994, 2002). During 2008, however, these *Zostera* beds disappeared completely, leaving behind a bare muddy area (Cunha et al., 2013). Important changes in sedimentation dynamics were clearly observed during the last decade (Cunha et al., 2013) and have been identified as major drivers of seagrass loss elsewhere (Fourqurean and Rutten, 2004), but whether they are also at the basis of the disappearance of *Zostera* in the Mira remains unclear. During 2009, *Z. noltii* started showing slight symptoms of natural recovery characterised by growth pulses with an irregular spatial and temporal distribution of small-sized seagrass patches. Therefore, the spatial and temporal distribution of seagrass became strongly heterogeneous in both space and time (Cunha et al., 2013). This context of the seagrass collapse at the Mira estuary creates a unique opportunity to examine benthic faunal responses during the early natural recovery process of seagrass vegetation. Specifically, former data of the nematode assemblages from the Mira seagrass beds from two decades ago can be compared with data collected during the natural recovery process of seagrass.

This study aims to investigate changes in patterns of nematode density, diversity, and trophic composition between two distinct habitat conditions: “before” the collapse of seagrass beds, and during the early recovery “after” the seagrass habitat loss, through the analysis of: i) temporal and spatial distribution patterns of nematode communities, and ii) the most important environmental variables influencing the nematode assemblages. The following hypotheses were tested: i) there would be differences in nematode assemblage density, biodiversity and trophic composition during both ecological conditions, “before” and “after”; and ii) there would be differences in nematode assemblage density, biodiversity and trophic composition at different sampling occasions during both ecological conditions.

2. Materials and methods

2.1. Sampling area and design

Sampling was performed at the Mira estuary at the southwestern coast of Portugal (37°40′N, 8°40′W), a small mesotidal system with a semidiurnal tidal regime (amplitude 1–3 m during neap and spring tides, respectively). Together with its surrounding area, this estuary is included in a protected area, the Natural Park of “Sudoeste Alentejano e Costa Vicentina’. In the Mira estuary the depth is usually lower than 5 m, the width of the water is usually much less than 400 m, and the tidal influence extends to *ca.* 40 km upstream. The physical and chemical fluctuations mainly result from natural pressures due to the estuary's morphology. Upstream tidal penetration is generally limited by the region's annual rainfall distribution concentrated between January and March, with the rest of the year being usually dry. In addition, the annual rainfall has a clear influence on the changes of sedimentation dynamics (Paula et al., 2006). Due to the low, seasonal and limited freshwater input, the lower section of the estuary has a dominant marine signature, and was characterised until 2008 by extensive, homogeneous *Z. noltii* meadows mainly in the intertidal area, with a strong seasonality and high biomass in the warm months. Moreover, *Zostera marina* vegetation was also present before 2008 in the adjacent subtidal area (Cunha et al., 2013). In 2008, *Z. noltii* meadows disappeared completely, however, indications of natural recovery have been observed since 2009 (Cunha et al., 2013).

To compare the temporal and spatial distribution patterns of nematode communities both “before” the seagrass habitat loss and “after” the loss, during the early recovery period of seagrass beds, samples were collected at two sampling sites located in the intertidal sediments of the *Z. noltii* beds; Site A, *ca.* 1.5 km from the mouth of the estuary, and Site B, 2 km upstream from the river mouth (Fig. 1). Sampling collections were carried out at neap low tide, at the same sites, and following a similar seasonal pattern. The former data was sampled in June 1994, September 1994, December 1994, February 1995 and June 1995. During the early recovery period, samples were collected in February 2010, June 2010, September 2010, December 2010 and February 2011. Former data was sampled three times at each sampling occasion, separated by 15 days, and in each time two replicates were taken, with a total of six replicates. In view of the high spatio-temporal variability in seagrass cover, in the ‘after’ sampling, it was decided to collect all samples of a given sampling occasion at the same day, taking three replicates per station as in many other environmental impact studies focussing on the composition and diversity of estuarine or marine nematode communities (Adão et al., 2009; Alves et al., 2013).

2.2. Sampling and sample treatment

2.2.1. Biological data

Nematode samples were obtained by forcing hand cores (3.6 cm inner diameter) to a depth of 3 cm. All samples were preserved in 4% buffered formalin solution. Nematodes were extracted from the sediment using a density gradient centrifugation in colloidal silica (Heip et al., 1985). The fixed samples were rinsed on a 1000 μm mesh sieve followed by sieving on a 38 μm mesh. The fraction retained was washed and centrifuged three times using the colloidal silica polymer LUDOX HS-40 (specific gravity 1.18 g cm^{-3}). The supernatant of each washing cycle was again collected on a 38 μm sieve. After extraction, all nematodes were counted under a

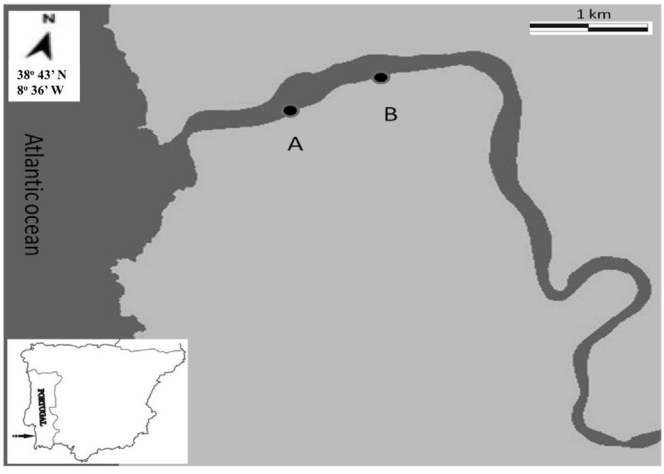

Fig. 1. Mira estuary (Portugal): indication of sampling sites (black circles) – (A, *ca.* 1.5 km from the mouth of the estuary, and B, 2 km upstream the river mouth).

stereomicroscope (40× magnification). A random set of 120 nematodes was picked from each replicate, transferred through a graded series of glycerol–ethanol solutions, stored in anhydrous glycerol, and mounted on slides (Vincx, 1996). Nematodes were identified to genus level using pictorial keys (Platt and Warwick, 1988) and the online identification keys/literature available in the Nemys database (Vanaverbeke et al., 2014). Nematode genus level is considered a taxonomic level with good resolution to discriminate disturbance effects (Moreno et al., 2008; Schratzberger et al., 2008; Warwick et al., 1990).

2.2.2. Environmental data

Salinity, temperature (°C), pH and dissolved oxygen (DO) (mg L^{-1}) of the overlying water above the sediment were measured *in situ*, using a WTW InoLab Multi 720 field probe. Additionally, at each site and sampling occasion, three replicate samples were collected from the water column and stored in bottles in the laboratory for future analysis of N and P nutrients (μmol L^{-1}) and chlorophyll *a* (mg m^{-3}). Nitrate ($NO_3^- - N$) and nitrite ($NO_2^- - N$) concentrations were analysed according to standard methods described in Strickland and Parsons (1972) and ammonium ($NH_4^+ - N$) and phosphate ($PO_4^{3-} - P$) concentrations were analysed following the Limnologisk Metodik (1992). Chlorophyll *a* (Chl *a*) determinations were performed according to Parsons et al. (1985). At each site and sampling occasion, three replicate sediment samples were taken randomly and stored in bottles to determine the organic matter content (OM) and grain size. OM was determined based on the difference between the dry weight of each sample after oven-drying at 60 °C for 72 h and the weight obtained after combustion at 450 °C for 8 h, and was expressed as the total weight %. Grain size of the sediments collected was analysed by dry mechanical separation through a column of sieves of different mesh sizes. The following size categories of sediment were determined: the amount of clay (<4 μm), the amount of silt (between 4 and 63 μm) and the amount of sand (>63 μm). The relative content of the different grain size fractions was expressed as a percentage. *Z. noltii* was sampled randomly at each site and sampling occasion. Three replicate samples were taken at each site before and three after the collapse, using sediment hand-corers with a surface area of 141 cm^2 and 30 cm in depth. Roots and leaves were separated and oven-dried at 60 °C for 48 h, then leaf and root biomass was estimated by the organic weight and the ash-free dry weight (gm^{-2} − AFDW). AFDW was obtained as the weight loss of the dry material after combustion at 450 °C for 8 h in a muffle furnace (Heraeus KR 170E).

2.3. Data analysis

Univariate and multivariate analyses were performed to detect temporal and spatial changes in the nematode community structure between “Sites” and “Sampling occasions” under two ecological conditions: mature seagrass vegetation “Before” and scattered and unstable seagrass patches “After” habitat loss. The statistical analyses of biological and environmental data were performed using the PRIMER v6 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008).

2.3.1. Environmental variables

A Principal Component Analysis (PCA) of the environmental variables measured was performed to explore patterns in multidimensional data by reducing the number of dimensions with minimal loss of information. The PCA ordination was based on the values of each environmental variable measured “Before” and “After” the habitat loss, by “Sites” and “Sampling occasions”. Prior to the calculation of the environmental variables’ resemblance matrix based on Euclidean distances, data were checked for uniform distribution, and if necessary were log (X + 1) transformed and normalized (subtracting the mean and dividing by the standard deviation, for each variable) prior to analysis. Selective log (X + 1) transformations were required for the water environmental variables, Chlorophyll *a*, nitrate, nitrite, ammonium and phosphate concentrations.

2.3.2. Nematode assemblages

Total nematode density (individuals 10 cm^{-2}), genus composition, trophic composition and several ecological indicators, either based on diversity: Margalef’s richness Index (*d*) (Margalef, 1958) and Shannon–Wiener diversity (*H′*) (Shannon and Weaver, 1963), or on ecological strategies: Index of Trophic Diversity (*ITD*) (Heip et al., 1985) and Maturity Index (*MI*) (Bongers, 1990; Bongers et al., 1991), were calculated using the nematode dataset from each site and sampling occasion, from both “Before” and “After” the habitat loss. In order to investigate the trophic composition of the assemblages, nematode genera were assigned to one of the four feeding groups designated by Wieser (1953), mainly based on mouth morphology: selective (1A) and non-selective (1B) deposit feeders, epigrowth feeders (2A) and omnivores/predators (2B). Based on this feeding-type classification, the Index of Trophic Diversity (*ITD*) was calculated as the sum of the squared proportional abundances of each feeding type (Heip et al., 1985), and its reciprocal (ITD^{-1}) was used, so that the higher values of the index correspond to higher trophic diversity.

The Maturity Index (*MI*) (Bongers, 1990; Bongers et al., 1991) was used as a measure of nematode life strategy. Nematode genera were assigned a value on a colonizer–persister scale (*c–p scale*) from 2 (colonizers) to 5 (persisters), where taxa with characteristics of good colonizers, such as rapid growth and production of many progeny, usually also characterised by a high tolerance to disturbance are considered colonizer; whereas persisters are slow-growing and often more sensitive taxa which thrive well in fairly stable and pristine environments (Bongers, 1990; Bongers et al., 1991). *MI* is calculated as the weighted average of the individual colonizer–persister (*c–p*) scores as $MI = \sum_{i=1}^{n} v(i) \times f(i)$, where $v(i)$ is the *c–p* value of the taxon *i* and f(*i*) is the frequency of that taxon.

A two-way permutational analysis of variance (PERMANOVA) was applied to test the hypothesis that significant differences existed between “Before” and “After” habitat loss, among “Sampling occasions” and “Sites” in nematode assemblage descriptors: total density, genera diversity and trophic composition, and the indices *d*, *H’*, *ITD* and *MI*. The PERMANOVA analysis was carried out following the three factor design: “Time”: Before and After (2 levels, fixed); “Site”: A and B (2 levels, random) and “Sampling occasion”: June (1994, 1995 and 2010), September (1994 and 2010), December (1994 and 2010), February (1995, 2010 and 2011) (10 levels, random nested in “Time”).

Nematode density data were square root transformed in order to scale down the importance of highly abundant nematode genera and therefore increase the importance of the less abundant ones in analysis of similarity between communities. The PERMANOVA analysis was conducted on a Bray–Curtis similarity matrix (Clarke and Green, 1988). The null hypothesis was rejected at a significance level <0.05 (if the number of permutations was lower than 150, the Monte Carlo permutation *p* was used). Whenever significant interactions in effects of the factors were detected, these were examined using *a posteriori* pairwise comparisons, using 9999 permutations under a reduced model. The similarity in communities in Time (before and after), Sampling occasions and Sites were plotted by Principal coordinates analysis (PCO) using the Bray–Curtis similarity measure. The relative contribution of each

genus to the average dissimilarities between Time, Sampling occasions and Sites was calculated using the two way-crossed similarity percentage analysis (SIMPER, cut-off percentage: 90%). The relationships between multivariate community structure and environmental variables were examined using the BIOENV procedure (Clarke and Ainsworth, 1993), which calculates rank correlations between a similarity Bray–Curtis matrix derived from biotic data and Euclidean distance matrices derived from various subsets of environmental variables, thereby defining suites of variables which 'best explain' the biotic structure (Somerfield et al., 1994). This procedure was done using Spearman's rank correlation coefficient (ρ).

3. Results

3.1. Environmental variables

Based on the results of the environmental variables measured, opposite trends were shown between the "Before" and "After" habitat loss conditions. The biomass of *Z. noltii* before the collapse was much larger than after habitat loss (Fig. 2). The PCA ordination of the environmental variables showed that the first two components (PC1, 52.7% and PC2, 15.6%) accounted for 68.2% of the variability of the data (Fig. 3). PCA ordination separated samples collected before the vanishing of the seagrass bed from the samples during the early recovery process of the ecosystem, mainly due to the highest values of *Z. noltii* biomass, ammonium concentrations and dissolved oxygen in the water, and organic matter in the sediment before the collapse of *Z. noltii*. The samples from early recovery were characterised by a higher percentage of sand in the sediment and higher values of nitrate and nitrite in the water. In addition, the samples of the "summer" months (June and September) were clearly separated from those of the "winter" months (February and December), particularly due to the higher values of chlorophyll *a*, temperature and salinity in the "summer" months.

3.2. Nematode assemblages – density

Significantly ($p < 0.05$) higher densities of nematodes were detected before the collapse than after habitat loss (Table 1). Before the seagrass collapse, the mean density ±SE was 1798 ± 180 ind. 10 cm^{-2} at Site A and 3338 ± 517 ind. 10 cm^{-2} at Site B, and during the early recovery process after the collapse the mean density ±SE of nematodes was 1119 ± 147 ind. 10 cm^{-2} at Site A and 2819 ± 406 ind. 10 cm^{-2} at Site B (Fig. 4). In all sampling occasions the density of nematodes was consistently higher before the habitat loss at each site. PERMANOVA analysis also revealed significant differences in the factors "Sampling occasion" and "Site" ($p < 0.05$). Significant interactions between "Time" and "Site" and between "Site" and "Sampling occasion" were also revealed. In addition, a significant interaction occurred between all three factors ("Time" × "Site" × "Sampling occasion") ($p < 0.05$ for all; Table 2). Individual pairwise comparisons revealed low variability of densities between sites before the collapse with no significant differences (Pairwise Tests, $p_{A\ vs.\ B} > 0.088$), but in early recovery a significantly higher density of nematodes was observed at site B than at site A (Pairwise Tests, $p_{A\ vs.\ B} < 0.012$). In addition, individual pairwise comparisons also detected significantly higher nematode densities between sites "Before" the collapse than between sites "After" collapse (Pairwise Tests, $p_{A\ (Before)\ vs.\ A\ (after)} < 0.008$ and $p_{B\ (Before)\ vs.\ B\ (after)} < 0.008$, for Site A and Site B, respectively). These results are also supported by PCO ordination plot and clearly reflect a distinct pattern between "Before" and "After" conditions. Furthermore, this analysis confirms a low between-sites variability of nematode densities before the habitat loss, whereas sites A and B were clearly separated during the early recovery phase (Fig. 5).

3.3. Nematode assemblages- structural diversity

Overall, 58 nematode genera belonging to 21 families were identified from the before-collapse period (from 1994 to 1995). Most genera belonged to the orders Monhysterida (51.4%), Chromadorida (44.9%) and Enoplida (3.7%); the dominant families were Linhomoeidae (35.0%), Desmodoridae (19.7%), Comesomatidae (16.1%) and Axonolaimidae (10.9%). The five genera *Terschellingia* (26.8%), *Paracomesoma* (15.4%), *Spirinia* (14.2%), *Odontophora* (10.8%) and *Linhomoeus* (7.2%) together comprised nearly 75% of the total nematode density and fifteen genera accounted for 90%. During early recovery, identified nematodes belonged to 50 genera and 22 families. Most genera again belonged to the Monhysterida (48.2%), Chromadorida (47.1%) and Enoplida (4.7%); the dominant

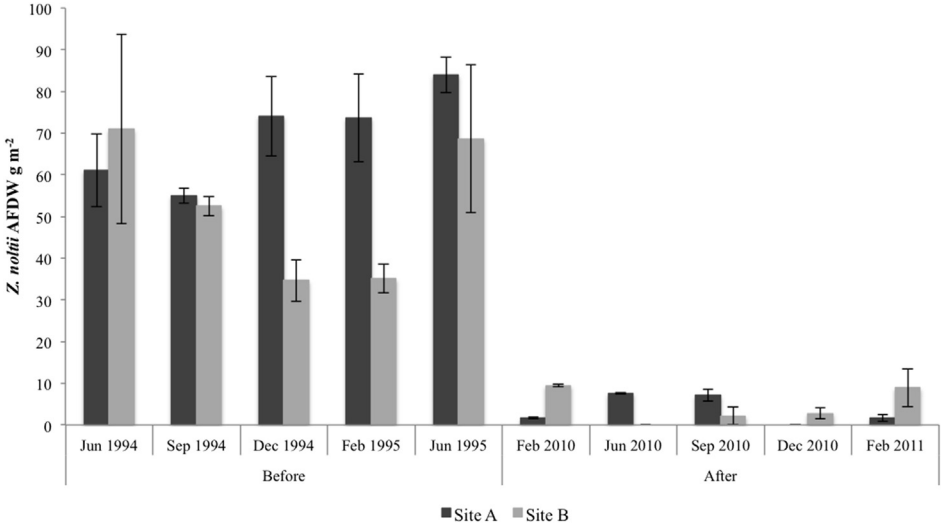

Fig. 2. Mean *Z. noltii* biomass ± standard error (SE) as AFDW (ash free dry weight) (g m^{-2}) at each sampling occasion (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011), site (A and B) and before and after the collapse of *Z. noltii*.

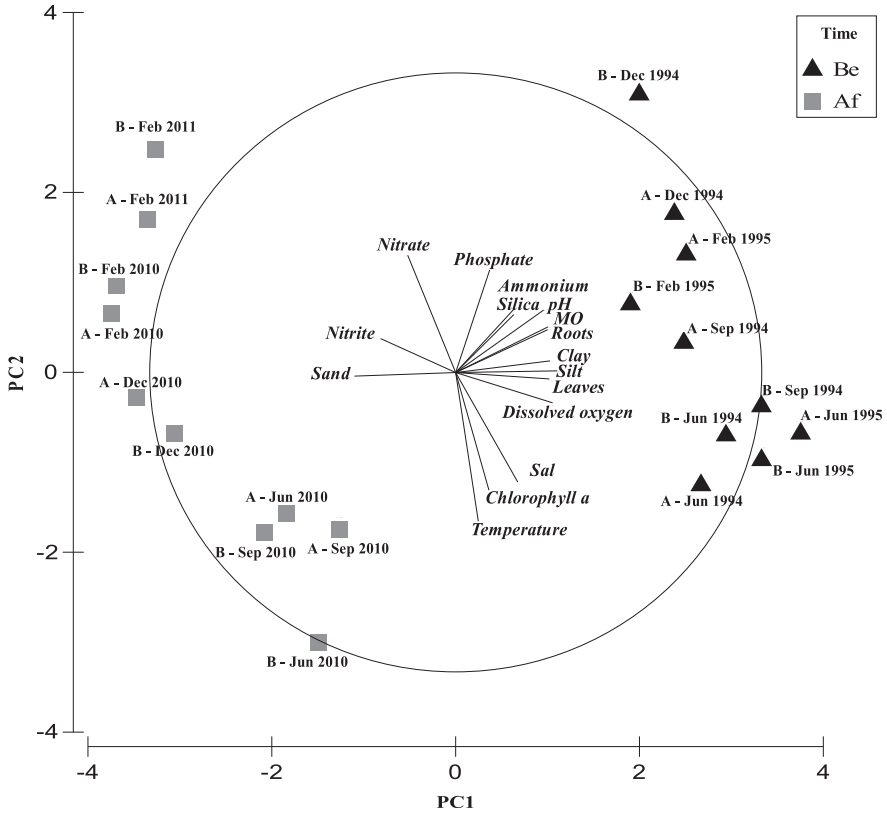

Fig. 3. Principal Component Analysis (PCA) plot based on the environmental variables measured at each "Time" (before and after collapse, 2 levels, fixed), "Site" A and B (2 levels, random) and "Sampling Occasion" (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011) (10 levels, random, nested in "Time"). PC1 = 52.7%, PC2 = 15.6%.

families were Linhomoeidae (29.1%), Comesomatidae (20.6%), Axonolaimidae (10.8%) and Desmodoridae (10.8%). Nine genera, *Terschellingia* (19.6%), *Paracomesoma* (14.6%), *Odontophora* (8.5%), *Ptycholaimellus* (6.1%), *Spirinia* (6.0%), *Sabatieria* (5.3%), *Linhomoeus* (5.1%), *Metachromadora* (4.8%) and *Daptonema* (4.7%) together comprised nearly 75% of the total nematode density and sixteen genera accounted for 90%.

The SIMPER analysis showed how nematode genera contributed to the similarity values of *a priori* defined groups, before and after habitat loss. From the total of 70 genera identified before and after the collapse, 38 were common in both situations and 32 were not present in at least one of the two groups. The genera *Terschellingia*, *Paracomesoma* and *Odontophora* presented the highest contribution to the similarity at both "Before" and "After" the habitat loss groups (Table 3). The genera *Chromadorella*, *Chromadora* and *Paramonhystera* contributed to the similarity "Before" the collapse (Table 3 – A), however they were absent "After". In contrast, the genera *Axonolaimus*, *Promonhystera*, *Anoplostoma* and *Dichromadora* contributed to the similarity during the recovery but were absent before the collapse (Table 3 – B).

Before the collapse, the mean number of genera (±SE) was 14.4 ± 0.4, and after collapse it was 20.1 ± 0.5. PERMANOVA showed significantly higher values of number of genera "After" the collapse (factor Time, $p < 0.05$). No significant interaction was detected for the number of genera between the three factors ("Time" x "Site" x "Sampling occasions", $p > 0.05$) between "Time" and "Site", and between "Site" and "Sampling occasion" (Table 2).

Genera richness and structural diversity based on Margalef Index (d) and Shannon–Wiener values (H') showed significantly higher values ($p < 0.05$) after the collapse. However, no significant differences were observed for the other factors "Site" ($p > 0.05$) and "Sampling occasion" ($p > 0.05$) (Fig. 6 and Table 2). No significant interaction was detected between the three factors ("Time" × "Site" × "Sampling occasions", $p > 0.05$), between "Time" and "Site", and between "Site" and "Sampling occasion" for both indices (d and H') (Table 2).

3.4. Nematode assemblages- trophic composition

Trophic composition did not differ significantly ($p > 0.05$) between "Before" and "After" seagrass collapse (Table 2). Before the seagrass disappearance, nematode assemblages were characterised by high abundances (mean percentage ± SE) of epigrowth feeders (2A) (42.6 ± 4.6%) followed by selective deposit feeders (1A) (29.5 ± 5.3%), non-selective deposit feeders (1B) (22.4 ± 2.7%) and omnivores/predators (2B) (5.3 ± 0.9%). During the seagrass recovery process, nematode assemblages were characterised by high abundances of epigrowth feeders (2A) (34.2 ± 4.6%) followed by non-selective deposit feeders (1B) (33.3 ± 4.7%), selective deposit feeders (1A) (21.9 ± 4.3%), and omnivores/predators (2B) (10.4 ± 2.1%) (Fig. 7). PERMANOVA analysis showed significant differences of the nematode trophic structure between "Sites" ($p < 0.05$), and a significant interaction ($p < 0.05$) between all three factors ("Time" × "Site" × "Sampling occasions"). In addition, no significant interaction was detected for trophic composition between factors "Time" and "Site" ($p > 0.05$), nor between factors "Site" and "Sampling occasion" ($p > 0.05$) (Table 2). Individual pairwise comparisons for the nematode trophic structure revealed low variability between Site A and B before collapse, with no significant differences (Pairwise Tests, $p_{A\ vs.\ B} > 0.152$), but a high variability between both sites during early recovery, with significantly higher values at site B (Pairwise Tests, $p_{A\ vs.\ B} < 0.022$).

Table 1
Mean density ± standard error (SE) of nematode genera (number of individuals per 10 cm^{-2}): A) before the collapse of *Z. noltii* on each sampling occasion and site. B) after the collapse of *Z. noltii* on each sampling occasion and site. Only the most abundant genera are included in this table.

A.	Before									
Genera	Site A					Site B				
	Jun 1994 ± SE	Sep 1994 ± SE	Dec 1994 ± SE	Feb 1995 ± SE	Jun 1995 ± SE	Jun 1994 ± SE	Sep 1994 ± SE	Dec 1994 ± SE	Feb 1995 ± SE	Jun 1995 ± SE
Terschellingia	638 ± 145	647 ± 177	274 ± 69	617 ± 141	58 ± 28	1031 ± 278	1074 ± 613	253 ± 102	2162 ± 765	229 ± 87
Paracomesoma	590 ± 209	494 ± 94	223 ± 52	643 ± 154	88 ± 71	456 ± 129	338 ± 139	180 ± 72	968 ± 221	65 ± 36
Spirinia	29 ± 5	90 ± 20	205 ± 46	158 ± 39	268 ± 106	482 ± 150	767 ± 162	262 ± 118	348 ± 215	966 ± 430
Odontophora	534 ± 181	198 ± 41	159 ± 55	385 ± 59	59 ± 20	547 ± 95	221 ± 39	119 ± 43	540 ± 87	191 ± 96
Linhomoeus	117 ± 51	67 ± 27	116 ± 37	133 ± 30	17 ± 6	350 ± 110	196 ± 58	86 ± 26	696 ± 268	95 ± 33
Daptonema	117 ± 41	18 ± 10	38 ± 14	162 ± 27	22 ± 11	141 ± 46	15 ± 8	36 ± 14	235 ± 55	141 ± 75
Metalinhomeus	3 ± 3	30 ± 27	0	6 ± 6	2 ± 2	0	0	0	0	0
Metachromadora	57 ± 22	16 ± 9	5 ± 5	22 ± 19	0	293 ± 112	22 ± 14	3 ± 2	152 ± 64	12 ± 5
Paracyatholaimus	0	20 ± 8	40 ± 15	143 ± 41	2 ± 2	12 ± 8	26 ± 20	34 ± 6	25 ± 11	10 ± 5
Sphaerolaimus	3 ± 3	49 ± 27	9 ± 9	3 ± 3	0	0	0	5 ± 3	84 ± 46	5 ± 5
Axonolaimus	0	0	0	0	0	0	0	0	0	0
Chromadorella	0	49 ± 20	250 ± 125	50 ± 13	17 ± 7	0	30 ± 11	22 ± 9	75 ± 27	6 ± 5
Viscosia	34 ± 26	14 ± 11	41 ± 15	36 ± 15	19 ± 9	65 ± 39	37 ± 12	32 ± 10	65 ± 21	52 ± 16
Dichromadora	0	0	0	0	0	0	0	0	0	0
Ptycholaimellus	7 ± 7	6 ± 4	5 ± 3	18 ± 12	12 ± 6	23 ± 23	10 ± 10	11 ± 7	97 ± 39	36 ± 20
Sabatieria	0	0	3 ± 3	0	0	0	0	0	0	4 ± 4
Atrochromadora	5 ± 5	0	0	0	0	0	0	0	0	0
Eleutherolaimus	11 ± 11	0	0	0	0	0	0	0	0	0
Oncholaimellus	0	10 ± 6	40 ± 31	4 ± 3	4 ± 3	5 ± 5	6 ± 6	18 ± 15	36 ± 36	14 ± 14
Desmodora	0	0	0	0	1 ± 1	421 ± 256	104 ± 104	2 ± 2	0	0
Molgolaimus	3 ± 3	3 ± 3	10 ± 5	20 ± 9	0	202 ± 114	6 ± 6	17 ± 14	186 ± 72	7 ± 7
Chromadorina	7 ± 7	5 ± 5	97 ± 76	42 ± 22	1 ± 1	0	43 ± 30	71 ± 37	77 ± 42	4 ± 4
Other genera	101 ± 96	157 ± 141	216 ± 145	185 ± 113	73 ± 60	496 ± 278	131 ± 94	126 ± 88	496 ± 435	181 ± 119
B.	After									
Genera	Site A					Site B				
	Feb 2010 ± SE	Jun 2010 ± SE	Sep 2010 ± SE	Dec 2010 ± SE	Feb 2011 ± SE	Feb 2010 ± SE	Jun 2010 ± SE	Sep 2010 ± SE	Dec 2010 ± SE	Feb 2011 ± SE
Terschellingia	180 ± 110	247 ± 184	280 ± 68	125 ± 25	71 ± 26	531 ± 225	728 ± 222	585 ± 419	948 ± 409	157 ± 45
Paracomesoma	64 ± 27	29 ± 11	453 ± 26	42 ± 16	81 ± 16	662 ± 54	336 ± 186	753 ± 244	350 ± 173	103 ± 17
Spirinia	269 ± 88	50 ± 1	316 ± 109	122 ± 23	64 ± 13	195 ± 79	82 ± 82	13 ± 13	35 ± 17	31 ± 28
Odontophora	98 ± 23	239 ± 196	220 ± 35	69 ± 31	92 ± 14	274 ± 54	210 ± 82	217 ± 77	243 ± 41	18 ± 4
Linhomoeus	31 ± 9	89 ± 27	50 ± 26	27 ± 1	13 ± 3	119 ± 44	134 ± 82	342 ± 231	145 ± 86	54 ± 18
Daptonema	47 ± 14	20 ± 7	34 ± 2	67 ± 21	70 ± 12	315 ± 127	113 ± 16	98 ± 39	82 ± 23	85 ± 36
Metalinhomeus	182 ± 80	58 ± 50	62 ± 26	58 ± 21	7 ± 7	46 ± 46	0	80 ± 40	111 ± 81	22 ± 22
Metachromadora	1 ± 1	16 ± 9	9 ± 9	5 ± 5	0	180 ± 180	93 ± 29	329 ± 127	291 ± 137	21 ± 13
Paracyatholaimus	19 ± 12	2 ± 2	0	29 ± 18	18 ± 5	371 ± 154	33 ± 25	30 ± 25	58 ± 21	33 ± 18
Sphaerolaimus	38 ± 23	66 ± 20	20 ± 5	14 ± 4	47 ± 6	42 ± 28	74 ± 29	109 ± 98	86 ± 26	32 ± 12
Axonolaimus	39 ± 16	89 ± 17	30 ± 10	10 ± 4	40 ± 15	53 ± 21	34 ± 19	76 ± 25	46 ± 10	35 ± 12
Chromadorella	0	0	0	0	0	0	0	0	0	0
Viscosia	51 ± 41	13 ± 13	0	11 ± 3	23 ± 13	21 ± 11	0	59 ± 19	34 ± 8	7 ± 7
Dichromadora	0	46 ± 25	0	0	0	0	147 ± 114	0	18 ± 8	3 ± 3
Ptycholaimellus	22 ± 19	57 ± 11	14 ± 9	65 ± 25	73 ± 16	194 ± 65	362 ± 235	190 ± 88	208 ± 101	18 ± 7
Sabatieria	7 ± 6	26 ± 13	11 ± 11	17 ± 9	9 ± 6	56 ± 30	168 ± 79	151 ± 45	479 ± 246	112 ± 20
Atrochromadora	72 ± 31	41 ± 31	19 ± 13	5 ± 2	4 ± 2	75 ± 14	149 ± 72	0	41 ± 30	14 ± 4
Eleutherolaimus	1 ± 1	14 ± 14	0	24 ± 24	2 ± 2	44 ± 31	34 ± 25	74 ± 4	42 ± 7	10 ± 5
Oncholaimellus	34 ± 16	33 ± 4	10 ± 5	15 ± 5	33 ± 7	21 ± 21	62 ± 25	4 ± 4	21 ± 15	2 ± 2
Desmodora	0	0	0	0	0	0	0	0	0	0
Molgolaimus	0	0	0	0	0	0	5 ± 5	0	0	0
Chromadorina	0	0	0	0	0	0	0	4 ± 4	0	0
Other genera	122 ± 92	79 ± 40	88 ± 54	77 ± 41	58 ± 45	334 ± 220	258 ± 207	202 ± 188	160 ± 129	69 ± 36

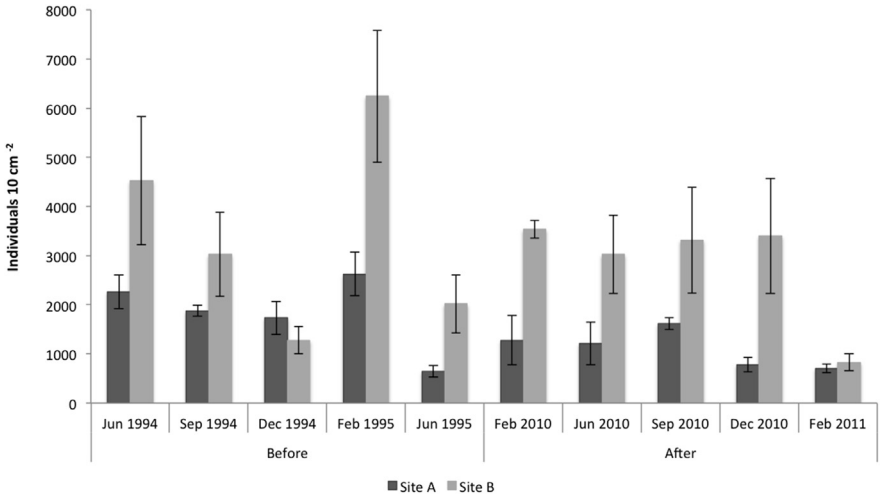

Fig. 4. Mean density ± standard error (SE) of nematodes (number of individuals per 10 cm^{-2}) on each sampling occasion (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011), at each site (A and B), and before and after the collapse of *Z. noltii*.

The index of trophic diversity (ITD^{-1}) before the collapse ranged from 1.84 ± 0.21 to 3.02 ± 0.17, and after the collapse from 2.78 ± 0.20 to 3.47 ± 0.26 (Fig. 8), but no significant differences were detected across all factors and their interactions ($p > 0.05$) (Table 2).

The Maturity Index (*MI*) ranged from 2.33 ± 0.03 (Site A, June 1994) to 2.66 ± 0.06 (Site B, June 1995) before the vanishing of the seagrass, and from 2.27 ± 0.07 (Site B, September 2010) to 2.59 ± 0.02 (Site A, February 2010) in the early recovery process. At both ecological conditions most nematode species showed a *c–p* score of 2 (before- 49.7%; after- 65.2%), described as 'general opportunists', or of 3 ('intermediate *c–p* group', before- 49.8%; after- 31.6%) (Fig. 8). Significant differences of *MI* among sampling occasions ($p < 0.05$) and a significant interaction of "Time" and "Site" were observed ($p < 0.05$). The individual pairwise comparisons of interaction factor did not detect any significant differences between Sites before and after the seagrass collapse (Pairwise Tests, $p_{A\ vs.\ B} > 0.072$ and $p_{A\ vs.\ B} > 0.212$, respectively).

Separate BIOENV analyses were performed for each habitat situation "Before" and "After" the collapse of *Z. noltii* in order to analyse the main factors responsible for the distribution of nematode communities. The BIOENV analyses showed a combination of six variables, each with a Spearman's rank correlation ($\rho = 0.5$), as the main factors more correlated with the nematode assemblage composition; organic matter (MO), root and leaf biomass of *Z. noltii*, oxygen percentage (O2), chlorophyll a (Chl *a*), silt and sand percentage.

4. Discussion

The natural recovery process of *Z. noltii* observed in the Mira estuary since 2009 created the opportunity to investigate changes on structural and functional composition of nematode assemblages as a response to ecosystem disturbance and habitat changes in an estuarine system. The "natural recovery" described by Elliott et al. (2007) implies a passive and ongoing process, which depends on improvement of structural and functional ecosystem quality. The causes of the *Z. noltii* collapse in the Mira are yet undetermined; the absence of visible anthropogenic pressures suggests a relation with natural stressors of this estuarine system, such as changes in annual river flow with direct consequences on sedimentation (Cunha et al., 2013; Vinagre et al., 2010). The significant decrease of the *Z. noltii* biomass has strong repercussions for various characteristics of the sediments, such as a decrease of the proportion of fine sediments and an increase of the sediment organic matter content. The presence of the seagrass beds enhances the proportion of finer sediments and food availability by trapping sediments, organic matter and nutrients due to the lower physical stress and water movements (Boström and Bonsdorff, 1997). Changes in the sedimentation dynamics have been observed in the Mira in the last decade, with an increase in sandy habitats, but this change preceded the seagrass loss (Cunha et al., 2013). The environmental variables before the *Z. noltii* disappearance showed a small range of values of temperature, salinity and chlorophyll a with a clear separation between summer and winter months. Nevertheless, temporal *Z. noltii* biomass changes were registered, with higher values in summer (June, September) and lower in winter months (December, February), in parallel with patterns from temperate and higher latitude waters (Duarte, 1989) and in agreement with earlier reports on seasonality of *Z. noltii* biomass from the Mira (Ferreira, 1994) and the Mondego estuaries (Grilo et al., 2012). After the habitat loss, the biomass of *Z. noltii* registered low values, with the absence of consistent temporal patterns being explained by the absence of stable seagrass patches. Instead, low-biomass patches continually emerged, disappeared and re-appeared at slightly different positions, creating a dynamic mosaic of vegetation patches interspersed with bare sediment patches.

As expected, the density of nematodes after the *Zostera* collapse was lower than before the collapse. This could be explained by the strong decrease of *Z. noltii* and by the increase of the proportions of coarser sediments. Many studies have reported that benthic organisms that live in seagrass beds have higher abundance, biomass, diversity and productivity (Boström and Bonsdorff, 1997; Edgar et al., 1994; Hemminga and Duarte, 2000; Hirst and Attrill, 2008; Webster et al., 1998), and in particular of nematodes (Alongi, 1987; Fisher and Sheaves, 2003; Guerrini et al., 1998) than unvegetated sediments. A higher variability of nematode density among sampling occasions was observed during 1994–1995 than during the early recovery process; this may be related to the higher values of *Z. noltii* biomass in the summer before the collapse. On the other hand, a higher temporal variability would be expected after the habitat loss due to the heterogeneous distribution of the *Z. noltii* after the collapse, but this was not the case. Furthermore, the increased *Z. noltii* biomass in the summer before the collapse could induce a seasonality in the nematode community density, as described for the intertidal temperate seagrass beds (Fisher, 2003),

Table 2
Details of the three-factor PERMANOVA test with "Time" (2 levels, fixed), "Site" (2 levels, random) and "Sampling Occasion" (10 levels, random and nested in "Time") for all variables analysed. Bold values highlight significant effects and interactions ($p < 0.05$).

	Source of variation	Degrees of freedom	Sum of squares	Mean squares	Pseudo-F	perms	P(perm)
Nematode total density	Time	1	22348	22348	3.2186	9949	**0.0365**
	Site	1	6691	6691	5.1211	9920	**0.0051**
	Sampling Occasion (Time)	8	27594	3449.2	2.5503	9891	**0.0002**
	Time × Site	1	4207.6	4207.6	3.2203	9928	**0.0115**
	Site × Sampling Occasion (Time)	8	10820	1352.5	1.5408	9803	**0.0008**
	Residual	66	57935	877.8			
	Total	85	1.29E+05				
Number of genera	Time	1	1475.7	1475.7	33.28	9962	**0.0002**
	Site	1	35.692	35.692	1.5893	9894	0.2377
	Sampling Occasion (Time)	8	360.29	45.036	1.9691	9955	0.1816
	Time × Site	1	4.2849	4.2849	0.19079	9911	0.6836
	Site × Sampling Occasion (Time)	8	182.97	22.871	1.2296	9944	0.2937
	Residual	66	1227.6	18.6			
	Total	85	3254.8				
Trophic composition	Time	1	1236.2	1236.2	0.76786	9949	0.6793
	Site	1	4045	4045	8.1192	9949	**0.0118**
	Sampling Occasion (Time)	8	10821	1352.6	2.5948	9951	0.0511
	Time × Site	1	1001.5	1001.5	2.0101	9948	0.1439
	Site × Sampling Occasion (Time)	8	4170.2	521.28	1.8429	9910	**0.0168**
	Residual	66	18669	282.86			
	Total	85	39456				
Margalef index							
	Time	1	1504.3	1504.3	22.485	9966	**0.0008**
	Site	1	2.8206	2.8206	0.12325	9915	0.7764
	Sampling Occasion (Time)	8	466.62	58.327	2.499	9960	0.1107
	Time × Site	1	15.155	15.155	0.66223	9918	0.435
	Site × Sampling Occasion (Time)	8	186.72	23.34	1.2524	9948	0.2828
	Residual	66	1230	18.636			
	Total	85	3346.2				
Shannon–Wiener index	Time	1	283.99	283.99	23.839	9973	**0.0004**
	Site	1	5.2678	5.2678	1.0179	9882	0.341
	Sampling Occasion (Time)	8	94.941	11.868	2.235	9955	0.1472
	Time × Site	1	1.3946	1.3946	0.26949	9907	0.6233
	Site × Sampling Occasion (Time)	8	42.478	5.3098	1.3553	9936	0.2369
	Residual	66	258.57	3.9178			
	Total	85	681.08				
Index of trophic diversity	Time	1	508.48	508.48	4.4808	9962	0.0603
	Site	1	0.24567	0.24567	6.14E-03	9916	0.9719
	Sampling Occasion (Time)	8	891.58	111.45	2.6773	9964	0.0918
	Time × Site	1	21.209	21.209	0.53005	9906	0.4969
	Site × Sampling Occasion (Time)	8	333.02	41.627	1.6687	9946	0.1181
	Residual	66	1646.4	24.946			
	Total	85	3476				
Maturity Index							
	Time	1	26.041	26.041	1.4658	9955	0.3393
	Site	1	0.89717	0.89717	0.4708	9862	0.5194
	Sampling Occasion (Time)	8	71.792	8.974	4.6385	9968	**0.0291**
	Time × Site	1	10.853	10.853	5.6949	9875	**0.0459**
	Site × Sampling Occasion (Time)	8	15.477	1.9347	1.1834	9953	0.3194
	Residual	66	107.9	1.6348			
	Total	85	238.25				

however no consistent temporal patterns were observed neither before nor after the *Zostera* collapse.

The number of nematode genera both before and after the habitat loss was generally high and comparable to those of the estuarine intertidal muddy sediments, commonly cited in the literature as mud-adapted (Smol et al., 1994; Soetaert et al., 1995; Steyaert et al., 2007) and typical of the intertidal sediments from the estuarine euhaline section in the Mira estuary (Adão et al., 2009). These assemblages are characterised by high densities of the genera belonging to the families Linhomoeidae (*Terschellingia*, *Linhomoeus*), Comesomatidae (*Paracomesoma*), Desmodoridae (*Spirinia*) and Axonolaimidae (*Odontophora*) (Austen and Warwick, 1989; Fisher and Sheaves, 2003; Fonseca et al., 2011; Johnson et al., 2007; Ólafsson et al., 2000; Rzeznik-Orignac et al., 2003; Smol et al., 1994; Soetaert et al., 1995; Steyaert et al., 2003; Tietjen, 1977; Wieser, 1960). Nematodes belonging to the aforementioned genera are often tolerant to hypoxic conditions (Jensen, 1984; Steyaert et al., 2007) and their slender bodies may be advantageous to glide through and over the fine sediments (Warwick, 1971), even though some papers have suggested that long slender bodies are more efficient to take up oxygen through diffusion (Fleeger et al., 2011; Soetaert et al., 2002). In this study, *Terschellingia* and *Paracomesoma* were the two most abundant genera registered both before and after the *Z. noltii* collapse; both are able to thrive in naturally and anthropogenically disturbed, oxygen-poor habitats (Alves et al., 2013; Armenteros et al., 2009; Gambi et al., 2009; Moreno et al., 2008; Steyaert et al., 2007). Their tolerance to low oxygen conditions may at least partly explain their prominence in seagrass beds.

In contrast to nematode density, structural diversity increased

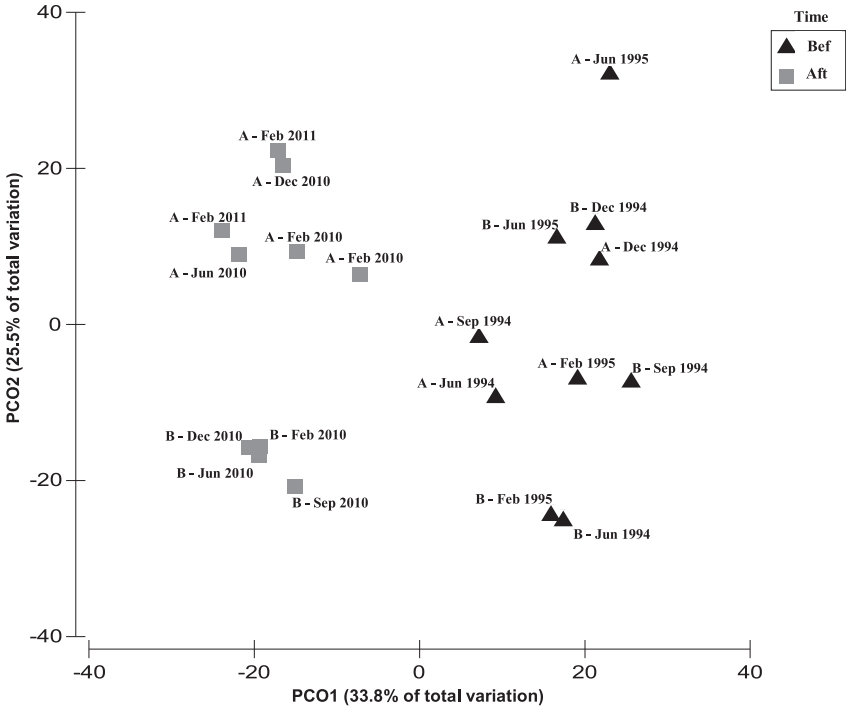

Fig. 5. Principal coordinates analysis (PCO) based on the nematode dataset before and after collapse ("Time", 2 levels, fixed), at each "Site" A and B (2 levels, random) and on each "Sampling Occasion" (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011) (10 levels, random nested in "Time"). PCO1 = 33.8%, PCO2 = 25.5%.

during the early habitat recovery. Although a majority of studies analysing meiobenthic communities associated with seagrasses have reported a higher diversity in vegetated sediments (Castel et al., 1989; Fisher and Sheaves, 2003; Guerrini et al., 1998; Ndaro and Olafsson, 1999), this has been contradicted by a few other studies (Fonseca et al., 2011; Ndaro and Olafsson, 1999; Tietjen, 1969). The dynamic sediment changes observed in the Mira estuary resulting in a higher proportion of coarse sediments may have contributed to the decrease of nematode density and the increase of diversity, because of the wider range of microhabitats available for nematodes in these sediments in comparison to muddy ones (Soetaert et al., 2009). Other authors have shown that the diversity of the nematode communities decreases in sediments with a high content of detritus and clay, but the abundance increases (Coull, 1985; Heip et al., 1985). The differences in nematode diversity were also explained by the presence and absence of several genera before and after collapse. Of the seventy genera identified in the present study, thirty-two were not present in at least one of the two ecological conditions. *Chromadorella*, *Chromadora* and *Paramonohystera* presented the highest contributions for similarity of the assemblages before the seagrass collapse but were absent after the collapse. *Axonolaimus*, *Promonhystera*, *Anoplostoma* and *Dichromadora* contributed strongly to the similarity during the early recovery but were absent before the collapse. Studies analysing nematode communities associated with vegetated and unvegetated sediments also detected differences partially explained by the presence and/or absence of several species restricted to seagrass or to unvegetated sediments, indicating that each site harbours a particular suite of species (Fonseca et al., 2011). Indeed, Fonseca et al. (2011) demonstrated that some nematodes found exclusively in unvegetated sediments presented morphological characteristics typical of coarser sediments that facilitate the active movement through the interstitial space and at the same time allow the nematodes to attach themselves to sediment grains to avoid suspension under hydrographic stress.

The trophic composition of nematode communities did not show significant differences between before and after the habitat loss. The epigrowth feeders (2A) were the most abundant trophic group of the nematode assemblages, in accordance with previous seagrass studies (Danovaro and Gambi, 2002; Ndaro and Olafsson, 1999; Tietjen, 1969). Diatoms and other microalgae are important food sources for many species of this trophic group (Moens and Vincx, 1997). Microphytobenthos (MPB) and epiphytic microalgae exhibit high production rates in seagrass beds (Danovaro et al., 2002; Fisher and Sheaves, 2003; Fonseca et al., 2011). MPB has been identified among the most important food sources for nematodes at both sampling sites in the Mira estuary during the recovery process (Vafeiadou et al., 2014), but even more so in bare intertidal sediments (Moens et al., 2005, 2014). Surprisingly, despite the low and unstable vegetation cover during the early recovery period, Vafeiadou et al. (2013, 2014) found highly similar resource utilization of macro- and meiobenthos in seagrass patches and in adjacent unvegetated sediments at the same study sites as in the present studies.

No temporal and spatial patterns emerged based on the Trophic Diversity Index (*ITD*) values before and after the collapse. *ITD* is generally used to correlate the trophic diversity of nematodes with pollution levels (Heip et al., 1985; Mirto et al., 2002), and statistically significant changes in this index can be obtained only when strong variations in the nematode assemblage structure occur (Moreno et al., 2008). The present results indicate that, despite the marked differences shown in environmental conditions, the nematode community maintained the feeding complexity with the presence of all trophic groups.

The low values of Maturity Index (MI) at both ecological conditions, suggest disturbed habitats since opportunistic genera with low MI values are dominant in disturbed and polluted environments (Gyedu-Ababio and Baird, 2006). Seagrass beds typically feature a dominance of genera with *c–p* score of 2 or 3 (Alves

Table 3
Genera that contribute most to the similarities and dissimilarities between communities before and after the collapse of *Z. noltii* as identified by SIMPER analysis: A) Distinguishing the genera present "before" and "after"; B) Distinguishing the genera present "before" or "after".

A.	Before	After	Before *vs* after	B.	Before	After	Before *vs* after
Genera	Similarity		Dissimilarity	Genera	Similarity		Dissimilarity
	49%	55%	59%				
Terschellingia	21.92	16.92	7.43	*Axonolaimus*	0	6.22	3.64
Paracomesoma	16.92	14.56	6.52	*Chromadorella*	4.99	0	2.81
Spirina	16.05	8.83	6.11	*Promonhystera*	0	2.48	1.41
Odontophora	15.05	11.68	4.5	*Chromadora*	2.51	0	1.33
Ptycholaimellus	2.8	9.19	4.13	*Paramonohystera*	1.98	0	1.1
Sabatieria	0.16	7.57	4.06	*Anoplostoma*	0	1.96	1.09
Linhomoeus	11.32	8.31	3.77	*Dichromadora*	0	1.92	0.99
Metachromadora	4.06	6.2	3.59	*Campylaimus*	1.4	0	0.74
Sphaerolaimus	1.68	6.35	3.47	*Megadesmolaimus*	1.2	0	0.73
Metalinhomoeus	0.52	5.86	3.33	*Desmodora*	1.58	0	0.66
Daptonema	7.62	8.73	3.12	*Elzalia*	0	0.93	0.52
Paracyatholaimus	4	5.21	2.78	*Paralinhomoeus*	0.81	0	0.44
Atrochromadora	0.08	4.8	2.61	*Southernia*	0.7	0	0.35
Viscosia	4.83	3.43	2.44	*Tricoma*	0	0.5	0.24
Oncholaimellus	1.71	3.87	2.26	*Eurystomina*	0.35	0	0.22
Chromadorina	3.45	0.11	1.89	*Odontanticoma*	0.34	0	0.22
Eleutherolaimus	0.12	3.41	1.84	*Thalassoalaimus*	0	0.32	0.19
Microlaimus	1.02	2.83	1.66	*Euchromadora*	0	0.25	0.14
Bathylaimus	2.37	1.53	1.53	*Crenopharynx*	0	0.33	0.13
Molgolaimus	3.14	0.13	1.47	*Onchium*	0.19	0	0.12
Halalaimus	0.24	2.34	1.4	*Paradesmodora*	0.31	0	0.12
Prochromadorella	0.68	2.07	1.33	*Desmolaimus*	0.17	0	0.11
Synonchiella	1.58	1.28	1.24	*Hypodontolaimus*	0.24	0	0.09
Camacolaimus	1.03	1.14	0.99	*Acanthoncus*	0	0.11	0.08
Paracanthonchus	1.41	0.5	0.89	*Antomicron*	0	0.14	0.07
Setosabatieria	0.86	1.01	0.86	*Chromaspirina*	0	0.08	0.05
Anticoma	1.19	0.15	0.79	*Neochromadora*	0.08	0	0.05
Oxystomina	0.63	0.86	0.76	*Phanodermopsis*	0.07	0	0.04
Cervonema	0.07	1.35	0.69	*Wieseria*	0.03	0	0.03
Comesa	0.37	0.73	0.6	Others	2.32	0	1.35
Thalassironus	0.52	0.39	0.52				
Leptolaimus	0.28	0.58	0.5				
Calyptronema	0.34	0.69	0.47				
Nemanema	0.22	0.64	0.42				
Diodontolaimus	0.46	0.1	0.33				
Cyartonema	0.19	0.41	0.32				
Aponema	0.07	0.43	0.21				
Aegialoalaimus	0.14	0.29	0.19				

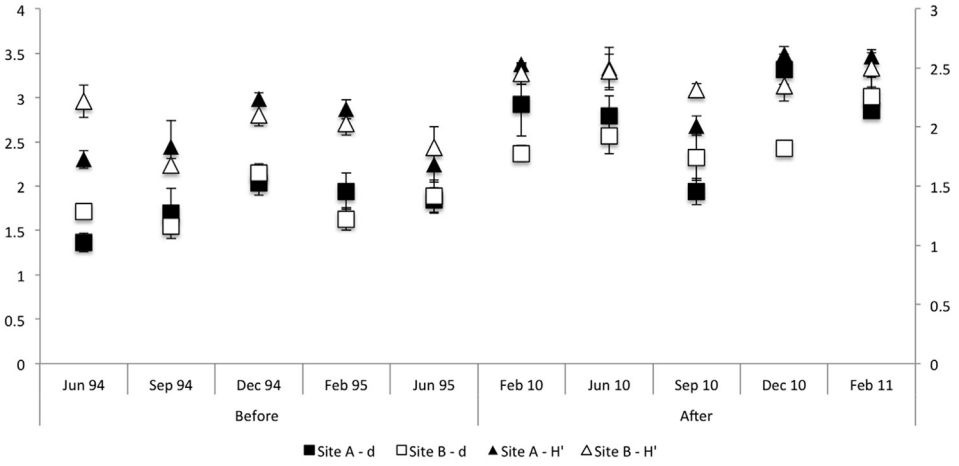

Fig. 6. Mean values ± standard error (SE) of Margalef Index (*d*) and Shannon–Wiener index (*H'*) at each sampling occasion (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011), site (A and B), and before and after the collapse of *Z. noltii*.

et al., 2013), probably because seagrass muddy sediments are naturally stressed due to the high degree of variability of the abiotic variables, particularly low oxygen. These sediments tend to have similar dominant nematode genera, which tolerate hypoxia and feed on the increased loadings of organic matter and their associated bacteria. Many of these dominant genera, such as *Terschellingia*, *Paracomesoma* and *Sabatieria*, have *c–p* 2 or 3, and they are described as indicators of a poor ecological quality status because of their well-known tolerance to pollution (Austen and Somerfield, 1997; Gambi et al., 2008; Schratzberger et al., 2006;

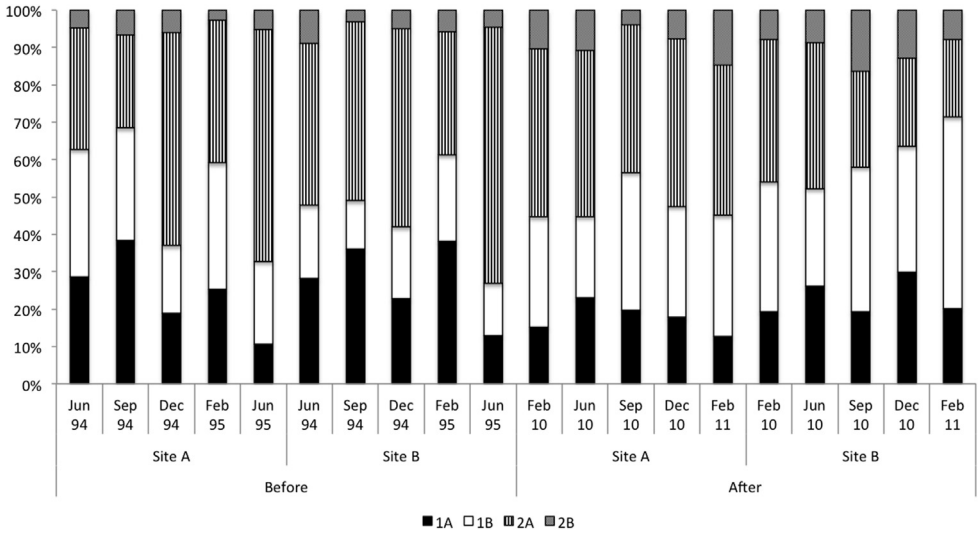

Fig. 7. Trophic group composition (1A – selective deposit feeders; 1B – non-selective deposit feeders; 2A – epistrate feeders; 2B – predators) at each sampling occasion (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011), site (A and B), and before and after the collapse of *Z. noltii*.

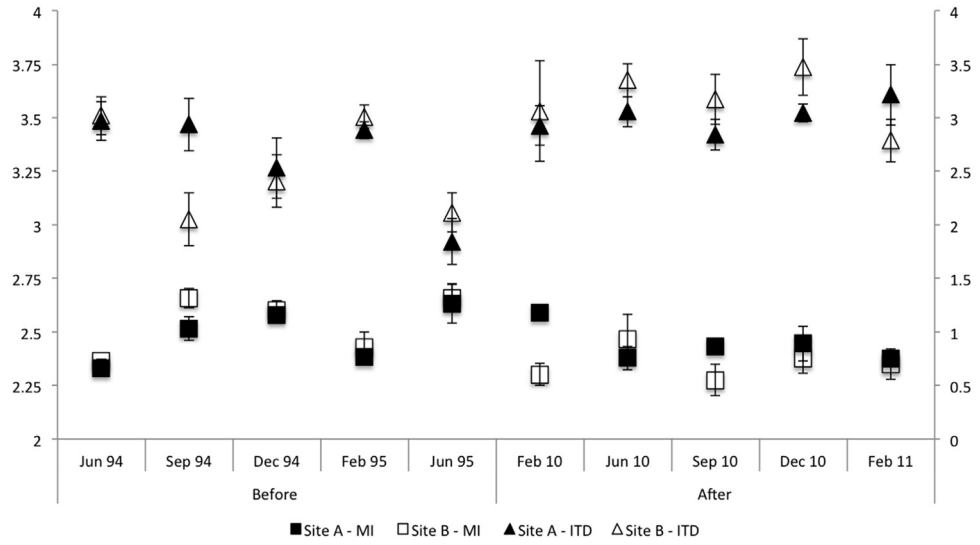

Fig. 8. Mean values ± standard error (SE) of the Index of Trophic Diversity (ITD^{-1}) and Maturity Index (MI) at each sampling occasion (June 1994, September 1994, December 1994, February 1995 and June 1995; February 2010, June 2010, September 2010, December 2010 and February 2011), site (A and B) and before and after the collapse of *Z. noltii*.

Soetaert et al., 1995; Steyaert et al., 2007). The *MI* index has been suggested to reveal some ambiguous results, distinguishing only the extreme conditions of disturbance (Moreno et al., 2011). The two ecological indices, Trophic Diversity (ITD) and Maturity Index (MI), behaved differently, indicating two important issues: first, a lack of sensitivity of these indices to habitat changes, since the two indices are seen as effective when strong variations in nematode assemblage structure occur (Moreno et al., 2011). Second, despite the great modifications caused by the collapse of *Z. noltii*, the early recovery process of this ecosystem continues to show typical seagrass bed characteristics that drive the nematode assemblage.

5. Conclusion

The present study shows that despite the disturbance caused by the seagrass habitat loss in the Mira estuary, the nematode assemblages revealed a high resistance. The nematode community features, both before and after seagrass vegetation loss, were typical of nematode assemblages of the estuarine euhaline section, which are well adapted to high stress conditions. The nematode community features investigated in this study suggest that meiobenthic ecological functioning has been maintained after habitat loss, although they have responded to changes on the ongoing process related to the *Z. noltii* seagrass bed natural recovery.

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