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

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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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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