



What makes a better indicator? Taxonomic vs functional response of nematodes to estuarine gradient

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ABSTRACT

Efficient implementation of nematodes-based indices for ecological quality assessment requires fundamental knowledge on their biodiversity and functional patterns along with the drivers that generate these patterns. Though, it is still unclear if nematodes taxonomical attributes are driven by the same environmental drivers as their functional (biological traits) counterparts, or if their taxonomical diversity is also enhanced by their functional diversity. To fill this knowledge gap, we investigated taxonomical (based on nematode genera abundances dataset) and functional attributes: trophic groups (TG) and life history strategies (LHS) of benthic nematodes collected from 35 sampling stations along the Sado Estuary, SW Portugal. Along with biological samples we measured environmental variables in the water and sediments as well as sediment grain size.

Our results demonstrated that taxonomy-based assemblages were mainly structured by the salinity gradient and further by the interplay of granulometry and organic matter content. Contrastingly, trait-based distribution patterns were largely driven by the variations in the above sediment dissolved oxygen concentration. This finding largely draw attention to the role that above sediment dissolved oxygen concentration exerts on nematode assemblages and their functional distribution patterns. Consequently, our results demonstrate that biological traits introduce a new dimensionality in multivariate data that otherwise could not be detected using solely taxonomical information, thereby enhancing our knowledge on ecological gradients existing within an estuary.

Additionally, we found a strong correlation between functional richness (based on the combination of TG and LHS traits) and diversity taxonomic metrics (species richness, Simpson and Shannon diversity), although no correlation was found between taxonomic diversity indices and single nematode ecological indices (ITD index of trophic diversity and MI Maturity Index). Therefore, the combined use of functional traits and its derived metrics was demonstrated to effectively reflect taxonomical diversity presenting reliable and highly complementary information for the assessment and monitoring of marine coastal sediments using benthic nematodes.

1. Introduction

Aquatic ecosystems undergo severe pressures from human induced activities causing unprecedented changes to community structure and affecting whole ecosystem function (Cardinale et al., 2012). In order to understand and mitigate the extent of these human induced activities in aquatic ecosystems, the Water Framework Directive WFD (WFD, 2000/60/EC) and recently also the Marine Strategy Framework Directive MSFD (MSFD, 2008/56/EC) implemented a series of strategic goals to achieve Ecological Quality Status (EcoQ) of coastal and inland waters and a Good Environmental Status (GES) of the European Seas by 2020. The EcoQ is assessed based on the composition, abundance and

sensitivity of different biological elements and significant scientific effort has been dedicated to the development of monitoring tools, including several benthic indices (Birk et al., 2012; Reyjol et al., 2014). Until now, macrofauna based indices have received the most scientific attention, particularly in estuarine and coastal ecosystems (Patrício et al., 2012). Recently, meiobenthic nematodes have also increasingly been used to assess the status of ecological quality but, despite the significant effort made to encourage their use as indicators of environmental conditions (Balsamo et al., 2012; Moreno et al., 2011), they are still not considered in the biological compartment of the European Directives framework.

Nematodes have been recognized as efficient indicators of

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environmental ecological conditions for several marine habitats, from the estuaries until the deep-sea ecosystems (Moreno et al., 2011; Semprucci et al., 2015). It is the combination of many attributes that gives them the status of a good bioindicator: (i) they are ubiquitous, and in high abundances and diversity; (ii) most nematode species have a short life cycle; (iii) some species/genera are tolerant to extreme conditions, and in addition, (iv) marine nematodes are easily sampled (Fonseca and Gallucci, 2016). Overall, nematodes are highly responsive to different types of pressures such as physical and chemical disturbances at spatial and temporal scales being an efficient tool for the assessment of the “Good Environmental Status” of marine ecosystems. The use of nematode assemblages to assess habitat condition revealed to be especially important in deep-sea sediment environments, where benthic nematodes represent the highest density from all of the metazoan taxa (Austen and Widdicombe, 2006; Höss and Traunspurger, 2003; Ramalho et al., 2018; Schratzberger et al., 2000). Additionally, the identification of nematodes is highly standardized through available existing electronic identification keys and molecular approaches to characterize nematodes from around the world (Avó et al., 2017; Bezerra et al., 2020; Tytgat et al., 2019). Despite these advantages, nematodes have received considerably little attention in the monitoring programs to assess the ecological status of coastal and marine environments, resulting in substantial knowledge gaps on their distributional patterns and drivers, particularly in the Iberian Peninsula.

The use of biotic indices for the assessment of ecological condition is largely based on the assumption that diversity patterns of a given community reflect the environmental conditions and, eventually those patterns can be linked to the ecosystem resilience (Gray et al., 2014). Traditionally, biotic communities have been characterized by their basic attributes such as: species richness, abundance or composition. However, those attributes can vary both spatially and temporarily, and do not always reflect the ecological role that the species play in the ecosystem (Heino, 2009; Sheaves, 2006). Moreover, changes in taxonomic attributes do not necessarily have to imply changes in the ecosystem stability (Friberg et al., 2011; Tyljanakis et al., 2010). Linkages between biodiversity and ecosystem function are therefore likely mediated by species traits, which determine the way organisms respond to abiotic environment (Bremner et al., 2003). In the recent decade functional trait-based approaches have been increasingly used to address several ecological-oriented questions, particularly to understand how different types of ecosystem disturbances affect species functional role (Franzo et al., 2019; Franzo and Del Negro, 2019) and related species functional diversity (Zhong et al., 2020). The growing need to measure the functional diversity, based on many available trait types, has prompted the development of functional diversity concept (FD) and several related indices (Carmona et al., 2016 and therein references; Mouchet et al., 2010; Villéger et al., 2008). These indices can be based on any type of traits (qualitative and quantitative) and return diverse functional metrics (i.e. functional richness, evenness, divergence and dispersion) that can be further compared to classical taxonomy-based indices to understand diversity-functional relationships of the communities (Zhong et al., 2020). FD indices have been demonstrated to have a high potential for Ecological Quality assessment (Mouillot et al., 2013) and have been recently successfully applied to assess functional changes of macrobenthic communities along natural gradients (Darr et al., 2014; Van der Linden et al., 2017; Liu et al., 2019), as well as to assess human driven impacts on the macrobenthic assemblages (Gusmao et al., 2016; Zhong et al., 2020). Despite these promising results, functional diversity approaches remain unexplored for the meiobenthic marine communities.

Nematodes present highly useful morphological and functional characteristics for the assessment of the “Good Environmental Status”, being considered the ideal model community to explore different aspects of diversity-function relationships in relation to the environment (Danovaro et al., 2008; Moreno et al., 2008; Fonseca and Gallucci, 2016). Except for their taxonomic attributes, nematodes possess

important functional characteristics such as trophic guilds (TG), based on mouth morphology (Wieser, 1956) and life history strategy (LHS), representing nematodes evolutionary adaptation to persist in a particular environment (Bongers et al., 1991; Bongers, 1999).

In conclusion, the efficient foundation for nematode-based indices for ecological status assessment requires fundamental knowledge on their biodiversity and functional patterns along with the drivers that generate these patterns (Bremner et al., 2006a, 2006b). Estuaries are an optimal case study to capture the highest spectrum of complex nematode assemblages–environment relationship, as they display a continuum gradient of environmental conditions (e.g. salinity, sediment granulometry, dissolved oxygen, temperature) triggering an adaptive assemblage response (Alves et al., 2009, 2013, 2014; Adão et al., 2009; Ferrero et al., 2008; Austen and Warwick, 1989). However, while the nematode’s taxonomical sufficiency in detecting spatial patterns is well acknowledged (Alves et al., 2009; Adão et al., 2009), little is known about nematodes functional response to the environmental changes (Franzo et al., 2019; Franzo and Del Negro, 2019). In fact, the relationship between nematode taxonomic features and functional traits and their efficiency for detecting spatial ecological conditions is still not fully established (Grzelak et al., 2016; Materatski et al., 2018; Schratzberger et al., 2007; Semprucci and Balsamo, 2014; Semprucci et al., 2018; Tita et al., 1999). Additionally, such studies have been so far limited to specific ecosystems i.e. harbours (Losi et al., 2013), deep sea (Leduc et al., 2013; Pape et al., 2013; Vanaverbeke et al., 2004; Vanreusel et al., 2010) and fjords (Grzelak, et al., 2016), remaining essentially unexplored along natural environmental gradients. Specifically, it is still unclear if nematodes functional attributes are driven by the same environmental variables as their taxonomical (community measures) counterparts (Schratzberger et al., 2007). To fill this knowledge gap our work aims to establish a link between environmental variables and diversity-function response of benthic nematodes throughout: 1) Determination of the spatial environmental gradients and related ecological niche information driven by the taxonomy-based assemblage distribution patterns and 2) Compare it with ecological information obtained through a traits-based assemblage approach along an estuarine gradient. 3) Examine if a higher taxonomic diversity enhances a higher functional diversity and if this relationship is consistent for either each trait separately or both traits combined.

Our hypothesis assume that drivers of taxonomical distribution will be strongly related with the environmental variables of the estuarine gradients, whereas traits-based functional responses will be sensitive to specific estuarine habitat conditions. Additionally, we hypothesize that taxonomic diversity will better reflect functional diversity when both traits (TG and LHS) will be considered in combination.

2. Methods

2.1. Study area

The Sado Estuary (38° 31′ 14″ N, 8° 53′ 32″ W) is the second largest estuarine system in Portugal, with an area of approximately 240 km² with a high socio-economic importance, supporting urban areas and important industrial and harbour-associated activities (Caeiro et al., 2005). The upstream areas are intensively explored with rice fields, although most of the estuarine area is classified as a protected area, designated as “National Reserve of the Sado Estuary”. The Sado Estuary has a semi-diurnal mesotidal system with tidal amplitude varying between 1.6 m and 0.6 m during spring and neap tides, respectively. Salinity is influenced by the Sado river flow (annual mean of 40 m³.s⁻¹) changing with seasonal and inter-annual conditions (Gonçalves et al., 2015), and with temperature ranging from 10 to 26 °C. This system is partially separated by intertidal sandbanks (*Troia beach*) and linked to the ocean by a 50 m deep channel (Gonçalves et al., 2015). The intertidal area has approximately 78 km² and 30% of this area consists of salt marshes and intertidal flats (Lillebø et al., 2011).

2.2. Sampling methods

A sampling survey was performed in the Sado Estuary during May of 2018. Nematode assemblage samples were collected at 35 sampling stations along the estuarine gradient that included: upstream stations, with river influence, middle estuary characterized by mesohaline salinity conditions, downstream euhaline stations, closer to the estuary mouth, and bay that presents the lowest hydrodynamics and the highest water residence time (Fig. 1).

Salinity, temperature ($^{\circ}\text{C}$) and dissolved oxygen (DO) (mg L^{-1}) were measured near the bottom at each sampling station using a multiparameter probe (YSI Data Sonde Survey 4). Sediment samples (~ 100 g) were collected using a grab, to determine total organic matter (TOM) and sediment grain size. Sediment samples were oven dried for 72 h at 60°C and subsequently combusted at 550°C for 8 h. TOM was calculated as the difference between the total weights of dry sediment and inorganic portion of sediment obtained through combustion. TOM was expressed as total % of organic matter. Sediment grain size was determined by sieving the collected sediments through a battery of different mesh sizes sieves. Grain sizes were assigned to five classes: gravel (>200 mm), coarse sand (0.5–2.0 mm), mean sand (0.25–0.5 mm), fine sand (0.063–0.25 mm) and silt&clay (<0.063 mm). All sediment fractions were expressed by the % of the total sediment weight (Brown and McLachlan, 2010).

Nematode assemblage samples were collected by forcing a hand core (10 cm^{-2}) into a depth of 3 cm into subtidal sediments collected by a Van Veen grab (0.05 m^2). The collected samples were preserved in a 4% buffered formalin solution. Fixed samples were first rinsed through a $1000\text{ }\mu\text{m}$ mesh and then through a $38\text{ }\mu\text{m}$ mesh. The retained fraction was washed and centrifuged three times, using colloidal silica polymer LUDOX HS-40 (specific gravity 1.18 g cm^{-3}) (Heip et al., 1985). All nematodes extracted were counted under a stereomicroscope Leica M205 C (100X magnification). A set of 120 nematodes was randomly picked from each sample, transferred through a graded series of glycerol-ethanol solutions, kept in anhydrous glycerol and placed on permanent slides (Vincx, 1996).

Nematode assemblages were identified until the genus level, using pictorial keys identification (Platt and Warwick, 1983, 1988; Warwick et al., 1998) and the online identification keys/literature available in NeMys database (Bezerra et al., 2020) were used. Nematodes were assigned to two functional traits:

- i. Trophic groups, where genera are assigned to four feeding groups, based on nematodes mouth buccal morphology (Wieser, 1956): selective deposit feeders (1A); non-selective deposit feeders (2B); epigrowth feeders (2A) and omnivores/predators (2B);
- ii. Life history strategies, where genera are assigned a value in a colonizer-persister scale (c-p scale), from 1 (colonizers) to 5

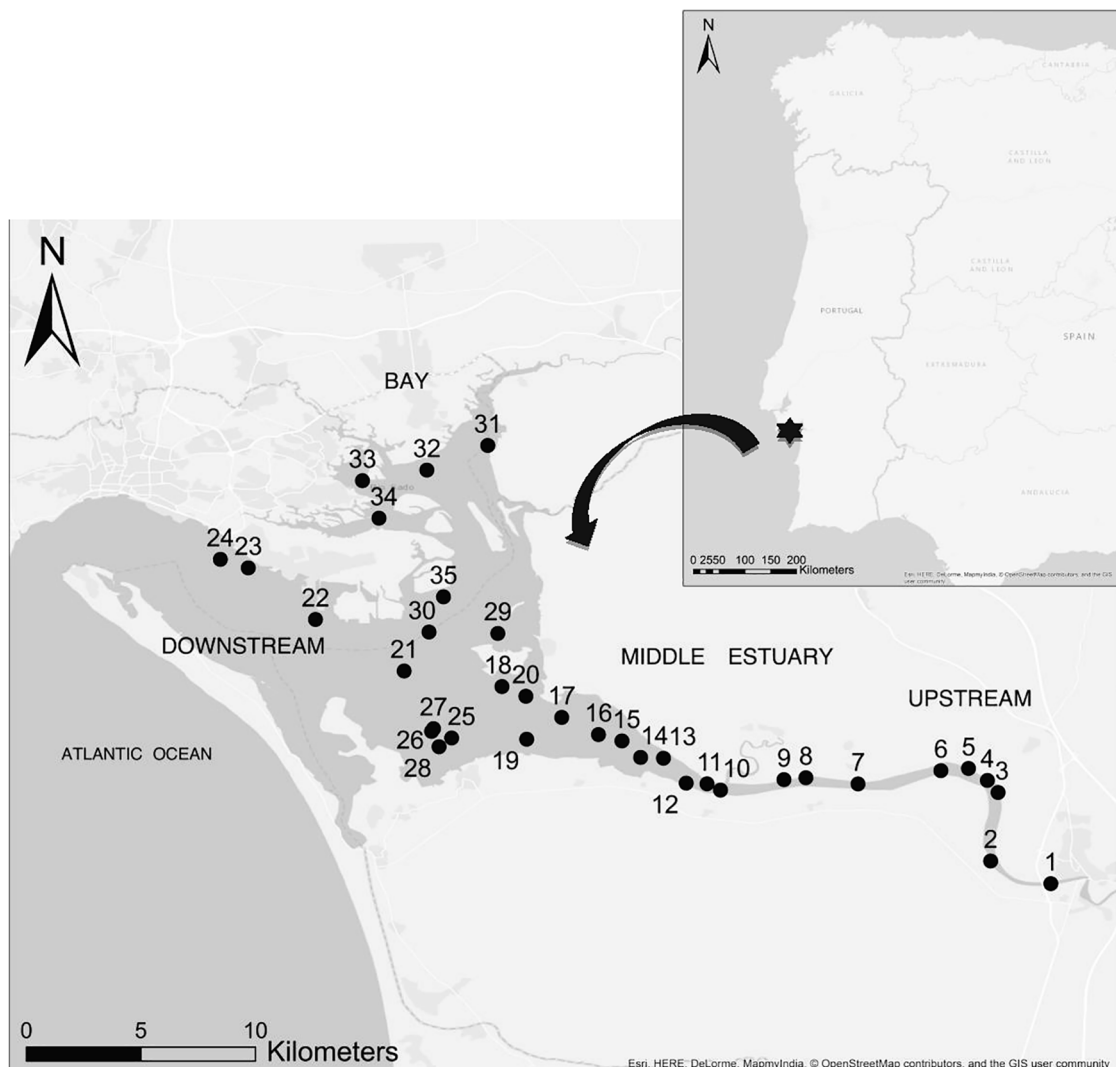


Fig. 1. Map of Sado Estuary with 35 sampling stations.

(persisters) (Bongers et al., 1991; Bongers, 1999). Colonizers are characterized by a rapid growth rate and reproduction and relatively high tolerance to disturbance. Contrastingly, persisters are characterized by a slow growth rate and are considered as sensitive to environmental change. Only c-p values from 1 to 4 were used in this study, as individuals assigned to c-p value 5 were absent from our samples.

2.3. Data analysis

2.3.1. Environmental variables

Natural distribution of environmental variables along the estuary gradient were visualized by performing principal component analysis (PCA) based on previously standardized environmental data and using PCA function of the FactoMineR package (Lê et al., 2008).

2.3.2. Environmental drivers of taxonomy-based nematode assemblages

Redundancy analysis (RDA) was conducted to test linear combinations of the environmental variables that best explain the variation of the taxonomy-based nematode assemblages. The response dataset consisted of Hellinger-transformed relative nematode genera abundance matrix (Legendre and Gallagher, 2001) and the explanatory matrix consisted of the environmental variables: pH, depth (m), temperature (°C), Dissolved oxygen (DO) (mg L^{-1}), DO (%), salinity, TOM (g), gravel (%), coarse sand (%), fine sand (%) and silt&clay (%). Variables were log10 transformed (pH, depth, temperature, DO (mg L^{-1}), salinity, TOM), except for sediment grain size and DO (%) variables, which were transformed using arcsine square root transformation. A forward selection procedure, using function `ordiR2step()` was used to select only significant variables ($p < 0.05$). Variation inflation factors (VIF) were calculated to check for linear dependencies and to ensure that only variables with small VIFs (< 10) were included. RDA analysis was performed in R (R Development Core Team, 2009) using “vegan” and “BiodiversityR” packages (Kindt and Coe, 2005; Oksanen, 2015). The effect of single explanatory variables on the assemblage composition patterns was analysed by variation partitioning of explanatory variables (Peres-Neto et al., 2006) using function `VarPart`. Four explanatory variables that were significant after forward selection were used: salinity, gravel %, TOM and pH. All the fractions were further tested for their significance using the “rda” function. The analysis was performed using function `varpart` in `vegan` (Oksanen, 2015).

2.3.3. Environmental drivers of traits-based nematode assemblages

Similarly, as described above, RDA was conducted on trait-based nematode assemblage datasets. Response matrix consisted of Hellinger-transformed: 1. Dataset where nematode abundances were aggregated by four functional trophic groups (1A, 1B, 2A, 2B); 2. Dataset where nematode abundances were aggregated by their c-p values (c-p 1, c-p 2, c-p 3, c-p 4).

2.3.4. Diversity and functional indices

Taxonomic diversity indices including species richness, Shannon entropy, Shannon diversity, Simpson diversity, and Pielou evenness were computed using function “diversity” of the `vegan` package (Villéger et al., 2008) for each sampling station.

Index of trophic diversity (ITD) (Heip et al., 1985) was calculated based on nematodes trophic guilds composition (1A, 1B, 2A and 2B) at each sampling station. ITD was computed as the sum of the squared proportion abundances of each of these feeding guilds, at each sampling station. Maturity index (MI) (Bongers et al., 1991; Bongers, 1999) was computed as the weighted average of the individual c-p scores and the individual taxon frequency at each sampling site.

Functional diversity indices were calculated based on two datasets: a sampling station by species table, and a species by functional traits table including two traits: trophic guilds (TG) and c-p values based on life-history strategies (LHS). Functional indices, based on the combination

of the latter two traits were computed using `dbFD` function of the `FD` package, based on Gower dissimilarity matrix from two trait types. The function returns the following functional diversity indices: Functional richness (FRic), functional evenness (FEve), functional divergence (FDiv) and functional dispersion (FDis) (Villéger et al., 2008). The mentioned indices capture different facets of assemblage functional trait structure and FRic, FEve and FDiv are considered analogous to taxonomic diversity metrics. FRic measures the amount of functional space (as a convex hull volume) filled by the community. FEve measures the evenness of species abundance distribution in a functional trait space. FDiv indicates how the abundance of species is spread along the functional trait volume. When the most abundant species are all clustered together in the functional trait range the FDiv is low. Whereas, when the most abundant species possess extreme trait values, being far from each other in the functional trait space volume, the FDiv is high. FDis measures the dispersion of traits in multivariate space also weighted by the genera abundances (Villéger et al., 2008).

The relationships between the diversity indices, sampling stations and environmental variables were examined by performing RDA, following a procedure described in 2.3.2 section. Response matrix was composed of taxonomic and functional diversity indices computed for each sampling station and explanatory dataset was composed of environmental variables. Linear mixed models were conducted to understand if higher taxonomic diversity indices enhanced higher functional indices. Functional diversity indices were used as response variables, and the taxonomic diversity as a fixed predictor. Where necessary, the variables were log-transformed. The best correlation results were represented graphically.

3. Results

3.1. Environmental parameters

Environmental variables measured at each sampling station along the Sado Estuary are provided in the Appendix 1. The salinity followed an estuarine gradient, registering progressively higher values from upstream oligohaline parts to middle estuary characterized by mesohaline salinity conditions. Downstream stations, close to the estuary mouth and bay presented euhaline conditions (Figs. 1 and 2).

The temperature values were similar along the estuary, from the highest value registered of 18.0 °C and the lowest of 15.5 °C. All the sampling stations generally presented a neutral pH (≈ 7) to slightly alkaline (8.2). Dissolved oxygen (O_2 mg L^{-1}) generally presented similar values along the estuary gradient averaging ≈ 7 –8 mg L^{-1} . The lowest value of 2.61 mg L^{-1} was registered at sample station S7 and the highest of 8.52 mg L^{-1} at station S11. The proportion of different sediment fractions and TOM content was highly variable within the sampling stations. In general, at the most upstream stations and those located next to the estuary mouth the proportion of coarser grain sediment was higher in comparison to middle estuary and bay, in which silt&clay fractions dominated.

First two PCA axes based on environmental variables in Sado Estuary explained over 60% of the total variability among sampling stations.

First PCA axis explained over 40% of the variability in the environmental data, and separated sampling stations according to the sediment type and organic matter content. Sand and silt&clay had the major contributions to the differentiation between sampling stations. Discrimination of the sampling stations according to the second PCA axis was driven by the differences related to salinity, pH and oxygen, from which pH had a major contribution to this ordination (Fig. 2).

3.2. Nematode assemblages

Overall, 96 nematode genera from 24 families and 6 orders were identified along the sampling stations of the Sado Estuary. Most genera belonged to the orders Chromadorida (63.3%) and Monhysterida

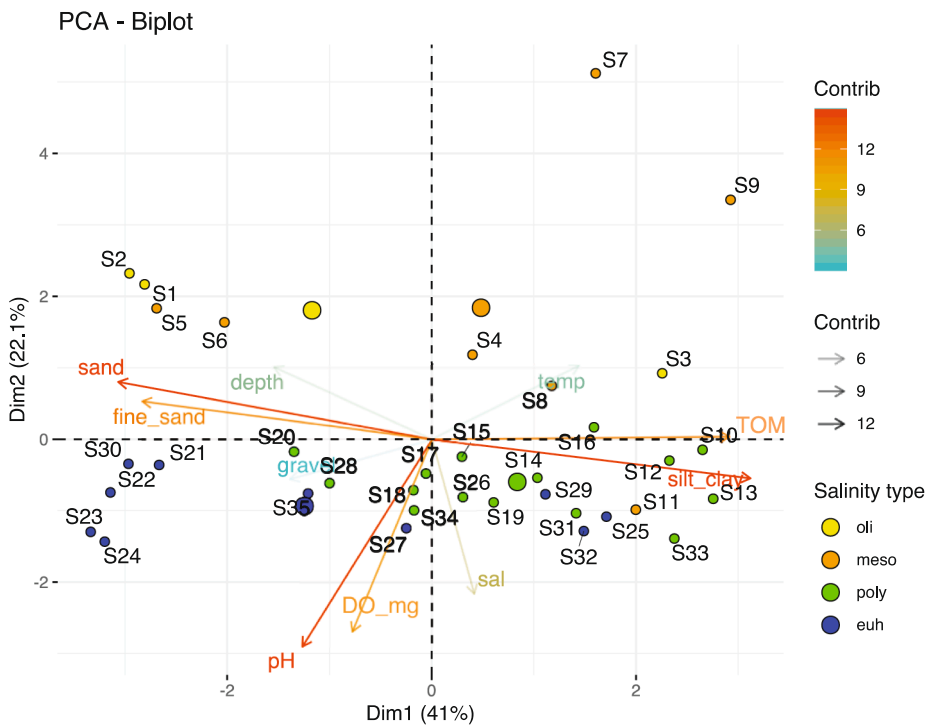


Fig. 2. PCA of the environmental variables with sampling stations according to salinity sections (oligohaline, mesohaline, polyhaline, euhaline) and environmental variables represented as vectors. Variable's vectors are presented by their contributions to the principal components (gradient colors and transparency of vectors) with red bright representing high contributions, yellow intermediate and transparent blue representing very low contributions. The numbers represent sampling stations.

(34.4%). The most abundant families were Comesomatidae (42.6%), Linhomoeidae (21.2%), Chromadoridae (9.04%), Desmodoridae (7.9%) and Axonolamidae (6.71%) representing 87.4% of the total families. Throughout the sampling stations, 6 genera accounted for 76.0% of total nematode density: *Sabatieria*, *Terschellingia*, *Paracomoesoma*, *Meta-chromadora*, *Parodontophora* and *Ptycholaimellus*. Mean nematode density in the estuary was 994.4 ± 241.3 ind. per 10 cm^2 , with minimum values at station S30 (9.3) and maximum at sampling station S31 (7271.6). The estuary area with the highest nematode density was bay (composed of stations S31, S32, S33, S34) with a total mean density of 4537.36 ± 1195.36 ind. per cm^2 . Sampling stations with the lowest nematode density were those located at the oligohaline upper estuary. The most abundant nematode genera per sampling station are presented in the Appendix 2.

3.3. Environmental drivers of taxonomy-based nematode assemblages

The RDA ordination on nematodes genera constrained by environmental variables was highly significant ($F = 3.0156$, $p = 0.001$, adjusted $R^2_{\text{Adj}} = 0.27$), with the first RDA axis explaining 0.15% and second explaining 0.11% of the variance in nematodes assemblage data. Groups of sampling stations separated along the first axis seem to be associated to the estuarine gradient, with the upstream locations, characterized by higher abundances of *Anoplostoma* and *Monhystrella* associated with lower salinity (oligohaline and mesohaline stations), while higher abundances of *Parodontophora* were associated to locations with higher salinity values (Fig. 3). Groups of stations separated along the second axis seem to be associated mainly to sediment grain size and dissolved oxygen concentrations, with higher abundances of *Molgolaimus*, *Metalinhomoeus* and *Rhabdodemania* occurring in locations with high proportion coarse sediments (gravel) and higher dissolved oxygen and pH. The third gradient is discriminated by sampling stations grouped in relation to organic matter content and temperature. The genus associated to stations characterized by high organic matter content and higher temperatures is *Terschellingia* genus (Fig. 3).

3.3.1. Variation partitioning (VarPart)

Variation partitioning demonstrated that overall environmental

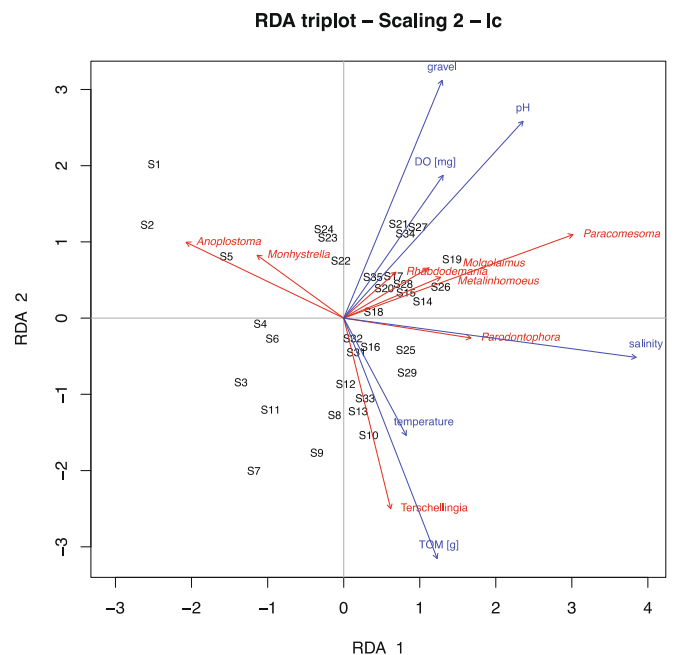


Fig. 3. RDA triplot drawn with the 'tripplot.rda' function with fitted site scores, on Hellinger transformed genera abundances data constrained by environmental variables. Only species with goodness of fit > 0.3 are displayed.

variables subjected to the analysis (salinity, gravel, TOM and pH) explained 25.4% of the variation in nematode assemblage composition, with 6.3% ('varpart', $F_{4.5288}$, $P < 0.001$) of the variation associated to salinity. The second most influential factor was the percentage of gravel fractions contributing to explain 4.3% ($F_{2.7639}$, $P = 0.005$). Further TOM explained 3.6% ($F_{2.7455}$, $P = 0.004$) of the variation and pH accounted for only 2.7% of the total variation explained ($F_{3.3558}$, $P = 0.001$).

3.4. Environmental drivers of traits-based nematode assemblages

RDA performed on the trophic group dataset was highly significant ($R^2_{Adj} = 0.30$, $F = 7.0726$, $p < 0.001$), with the first and second axis explaining 27% and 14% of the total variance, respectively. Nevertheless, the main gradients responsible for trophic group distribution patterns were DO (mg L^{-1}), DO (%) concentration and the presence of TOM. This pattern was particularly driven by the epigrowth feeders and predators (2A and 2B) that displayed positive affiliations to dissolved oxygen saturation and strong negative correlation with TOM content. Non-selective deposit feeders (1B) displayed their optimum at the conditions corresponding to the intermediate values of DO (mg L^{-1}) and organic matter content, (Fig. 4A). Deposit feeders (1A) were mainly

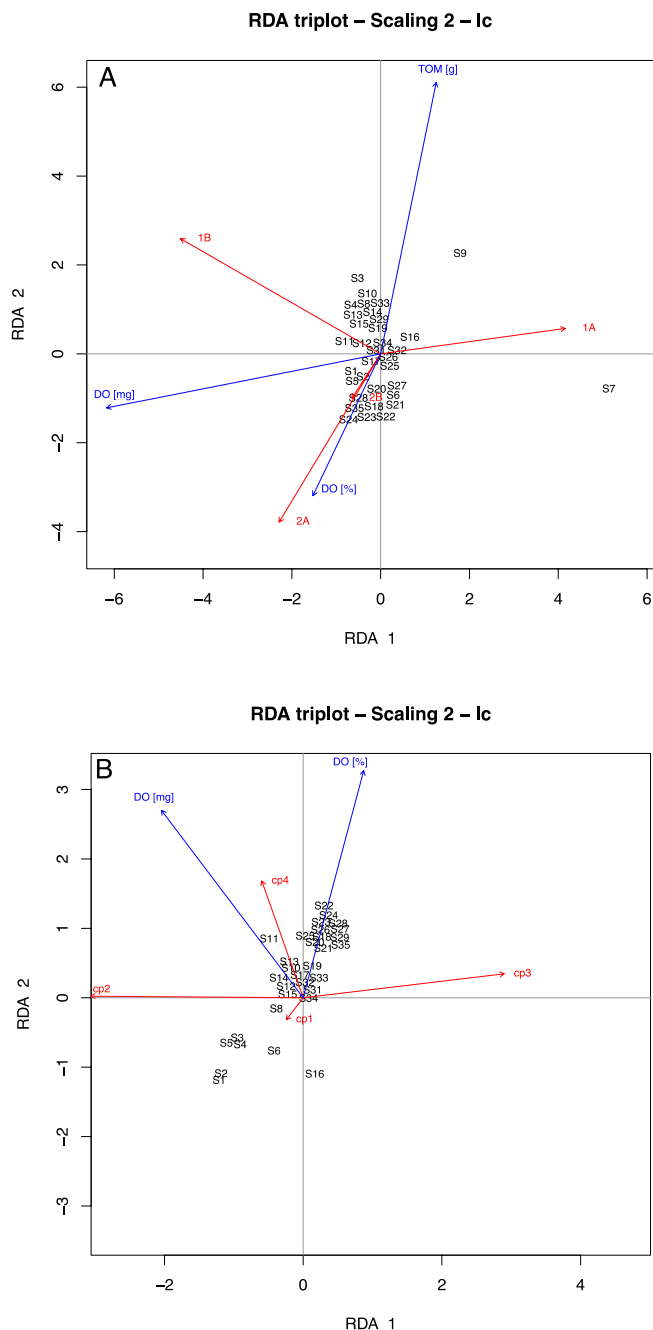


Fig. 4. RDA triplot on Hellinger transformed dataset a) Trophic group dataset, where genera were grouped by their trophic guilds, b) C-P dataset where genera were grouped by their c-p scores. Only genera with goodness of fit >0.3 are displayed.

associated to poorly oxygenated waters, being especially prevalent at stations: S7 and S9 with very low DO (mg L^{-1}) values DO (Fig. 4A). From all of the significant environmental variables dissolved oxygen (mg L^{-1}) contributed to most of the explained variation (25.50%) according to varpart analysis ($F_{9,4058}$, $P = 0.001$) and was responsible for stations grouping along the second axis. TOM (mg) contributed in 9.4% ($F_{4,4511}$, $P = 0.008$) and oxygen saturation contributed in 6.20%, although was not a significant component of a VarPart analysis. Both factors were responsible for the station discrimination along the first RDA axis.

RDA performed on c-p dataset was significant ($R^2_{Adj} = 0.34$, $F = 9.32$, $p = 0.001$). The first axis accounted for majority of the variability (32.33%) and the second axis explained only 5.22% variability among nematode c-p scores (Fig. 4B). The only two significant variables that discriminated the c-p score dataset along the first axis where DO (mg L^{-1}) and DO (%). Genera belonging to c-p 1 were clearly associated to upstream (freshwater) estuary sections characterized by the low DO saturation values. In contrast c-p 4 genera had high affinities to well oxygenated sampling stations (Fig. 4B). VarPart analysis demonstrated that only oxygen (mg L^{-1}) was a significant component ($F_{5,6901}$, $p = 0.034$) and contributed to majority of the variation (21.2%) in nematode c-p scores.

3.5. Environmental drivers of the taxonomy and functional diversity indices

Distribution of taxonomical and functional diversity indices discriminated among three different habitat conditions along an estuarine gradient (Fig. 5). First axis discriminated sampling stations located next to the estuary mouth characterized by high values of dissolved oxygen concentration and prevalence of fine sand. This type of habitat had the highest functional richness. On the opposite site there are sampling stations located mostly in the bay and downstream estuary dominated by the silt&clay fractions (negatively correlated with fine sand) and reduced oxygen concentrations. These sampling stations have

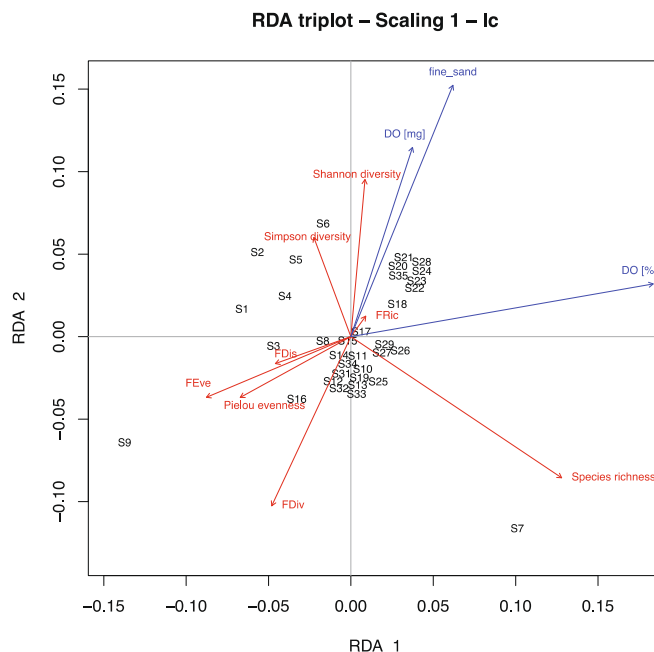


Fig. 5. RDA triplot on Hellinger transformed dataset composed of taxonomical and functional diversity indices constrained by the environmental variables. Abbreviations for the functional diversity indices are as follow: FRic – Functional Richness, FDis – Functional Dispersion, FDIV – Functional Divergence and FEve – Functional Evenness (Villéger et al., 2008). First axis explains 19.00% and second axis explains 16.68% of the variability among taxonomical and functional diversity indices. $R^2_{Adj} = 0.27$, $F = 5.7065$, $p = 0.001$.

the highest functional divergence, meaning that the most abundant genera at these stations have extreme functional trait values. The second RDA axis discriminated sites according to the saturation of dissolved oxygen. Similarly, the most exposed sampling stations were the ones with the highest dissolved oxygen saturation values, whereas stations with lower oxygen saturation had the highest taxonomical and respectively functional evenness, but also high functional dispersion. The highest species richness was at the sampling stations located near the estuary mouth (S26, S27, S29), whereas the most upstream oligohaline and mesohaline stations had the lowest species richness (Fig. 5).

Functional diversity of nematode assemblages increased as a function of species richness ($p < 0.001$, $R^2_{Adj} = 0.76$), but also Shannon diversity ($p < 0.001$, $R^2_{Adj} = 58$) and less with Simpson diversity ($R^2_{Adj} = 0.49$, $p < 0.001$) (Table 1, Fig. 6a,b,c). It is important to point out that ITD and MI indices alone were not significantly correlated ($P > 0.05$) with any of the taxonomical diversity metrics (number of species, Shannon and Simpson indices, Shannon entropy and Pielou evenness). Functional dispersion was also significantly and positively correlated with Shannon entropy ($R^2_{Adj} = 0.63$, $p < 0.001$), Simpson diversity ($R^2_{Adj} = 0.63$, $p < 0.001$) and Pielou evenness ($R^2_{Adj} = 0.80$, $p < 0.001$) (Table 1, Fig. 6 d,e,f).

4. Discussion

4.1. Environmental drivers of nematode assemblages distribution patterns

Our hypothesis was that the taxonomical drivers would be related to the spatial environmental factors of the estuarine gradients, whereas the functional responses would be more sensitive to small-scale variability in the estuarine habitat conditions. Our results only partially support this hypothesis, while also pointing out to the importance of different type of patch scale conditions in structuring taxonomy-based and trait-based nematode assemblages. Salinity and sediment grain size were found to be the main drivers of taxonomy-based distributional patterns along the Sado Estuary, supporting the importance of estuarine gradient in structuring nematode assemblages (Bowman, 1983; Adão et al., 2009; Alves et al., 2009, 2013; Coull, 1999; Ferrero et al., 2008; Schratzberger et al., 2008). However, while the salinity displayed a clear spatial gradient along the estuary, the sediment granulometry exhibited a rather patchy distribution. Consequently, the first gradient discriminated areas of marine versus freshwater influence and this discrimination was driven by the stenohaline marine and freshwater taxa. The second gradient was driven by the sediment granulometry where areas with coarser grain size (gravel) and high dissolved oxygen concentration and pH were clearly distinguished from more muddied sampling stations, where higher contribution of anaerobic process resulted in the lower concentration of dissolved oxygen (Steyaert et al., 2007). Additionally, a third gradient was identified, which differentiated areas based on the organic matter content, with the most opportunistic genera

(with lower c-p values) being associated to the sampling stations with high deposits of organic matter. Organic enrichment is known to be an important factor for meiofauna and nematode distributional patterns and similar findings were already reported in estuarine and marine habitat conditions (Bertocci et al., 2019; Kandratavicius et al., 2018; Schratzberger and Warwick, 1998) confirming this general trend. These three gradients were unrelated with each other, indicating that within a particular estuarine salinity area exist conditions characterized by the coarse/fine sediment types or high/low organic matter content inhabited by concomitantly divergent assemblages. As a result, environmental variables, other than salinity, produced spatially distinct areas rather than gradients on the sea floor.

While our study design did not permit to test the issue of scale dependency of the measured environmental variables, similar future study designs should account for the effect of hierarchical filtering for taxa turnover. Specifically, the hypothesis wherever salinity or the sediment properties acts as a major hierarchical filter that constrain the nematode distributional patterns (Menegotto et al., 2019).

When nematodes were bundled by their ecological strategies (trophic guilds and c-p scores) the main grouping factors that emerged were dissolved oxygen concentration and dissolved oxygen saturation (measured above sediment), demonstrating that these two factors participate in discriminating estuary areas in a different manner. The importance of the water dissolved oxygen concentration in the distribution patterns of the biological traits was also observed by Alves et al. (2014) when applying multi-trait analysis to nematode data in the Mondego Estuary, Portugal.

Meiofauna communities are highly affected by small-scale penetration of dissolved oxygen along the vertical sediment profile (Braeckman et al., 2013; Soetaert et al., 2002; Steyaert et al., 2007; Vanaverbeke et al., 2004). However, the effect of the above-sediment oxygen conditions on the nematode trophic guilds and life history strategies have not yet been addressed. Oxygen availability affects vital metabolic pathways in microbes as well as higher trophic level organisms, and it may indirectly influence nematode trophic guilds distribution patterns, throughout the interplay between organic matter content, bacterial decomposition and epibenthic photosynthesis (Rosenberg et al., 2002). On the other hand, dissolved oxygen levels might also limit macrofauna distribution patterns, thereby also conditioning nematodes functional composition through the trophic interactions (Magni et al., 2005; Mestdagh et al., 2018) and bioturbation (Bernard et al., 2019). The role of both forms of measured DO (as concentration and saturation) as proxies for underlying the processes affecting the distribution patterns of nematode trophic guilds and c-p scores undoubtedly deserves further investigation, as highlighted by our results.

Evidence drawn from the macrofauna research suggests that the biological traits structure the assemblages over smaller scales (local/habitat scales), while the taxon composition approach is more susceptible to identify broader scale patterns (Bremner et al., 2003; Bremner,

Table 1

Results of the linear mixed models used to test for the relationships between taxonomic and functional diversity of nematode assemblages in Sado Estuary, NW Portugal. The model degrees of freedom (df), Adjusted R^2 , model estimates with standard errors (SE) and P-values are given.

	Parameters	Source of variation	df	Adjusted R^2	Estimate (SE)	p-value
Taxonomic vs Functional Richness	Functional richness	N° of species	32	0.76	0.45 (0.04)	<0.001
		Intercept			−0.63 (0.12)	<0.001
	Shannon diversity	Intercept	32	0.58	0.33 (0.05)	<0.001
		Intercept			−0.07 (0.10)	0.502
		Simpson diversity	32	0.49	0.32 (0.06)	<0.001
		Intercept			0.07 (0.09)	0.491
Taxonomic vs Functional dispersion	Functional dispersion	Shannon entropy	32	0.63	0.35 (0.05)	<0.001
		Intercept			0.04 (0.03)	0.233
	Simpson diversity	Intercept	32	0.63	0.09 (0.01)	<0.001
		Intercept			0.15 (0.02)	<0.001
		Pielou evenness	32	0.80	−0.87 (0.04)	<0.001
		Intercept			1.12 (0.09)	<0.001

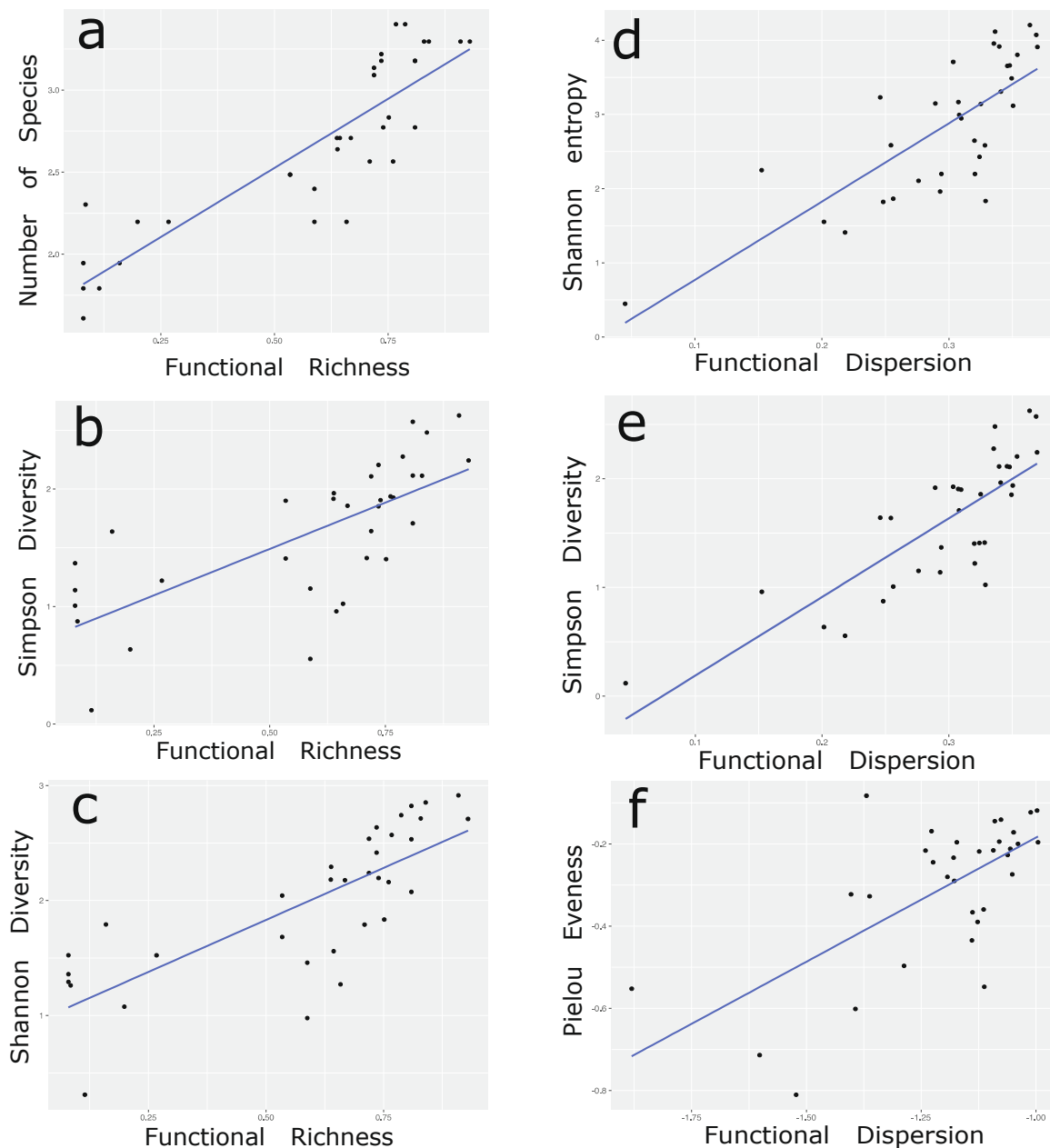


Fig. 6. Relationships of selected taxonomic (number of Species-a, Simpson Diversity-b, Shannon Diversity-c) indices and Functional richness as well as the taxonomic (Shannon entropy-d, Simpson diversity-e, Pielou Evenness-f) and Functional dispersion for nematode assemblages of Sado Estuary, SW Portugal. The indices of Functional richness and Functional dispersion were computed considering combined traits using “dbFD function” of the FD package (Villéger et al., 2008). Solid lines show the best-fitting linear mixed regressions of dependent variables. See Table 1 for the linear mixed model results (estimates and P-values) associated with the relationships between taxonomic and functional-diversity types. The number of species, Shannon, Simpson diversity indices and Pielou Evenness were log-transformed.

2008; Hewitt et al., 2008). For example, in the study of Bremner et al. (2003), assemblages based on different trait composition captured differences in ecological conditions on the scale of sampling stations, whereas, taxon composition revealed higher variability among geographical sectors.

We indeed found that small scale variability in both forms of oxygen impose a strong environmental constraint on taxa trait composition. Furthermore, oxygen variations among sampling stations were relatively small (i.e. no anoxic conditions were detected), which additionally strengthen the general concept that biological traits can identify higher heterogeneity among sampling stations as they are governed by environmental conditions acting on a smaller – habitat scale. It is worth to mention that previous research on this topic (Bremner, 2008; Hewitt

et al., 2008) was done at much larger geographical scales, suggesting that patterns might be independent on geographical dimensions and eventually can be also applied to identify ecological gradients within one estuary.

Interestingly, the variation explained by the environmental variables was much higher for functional traits ordination, than it was explained by the taxon distributions. In addition, this variability was explained by less number of environmental variables (three for trophic groups and two for c-p scores) suggesting that the variables responsible for ecological functions are more effective in capturing spatial differences than are the variables responsible for taxonomic ordination. A similar finding was also reported for macrofauna (Bremner et al., 2003) based on a Principal Component Analysis. This further indicates that there

might be general trends in nematode ecological functioning acting in parallel and at scales not detectable by the taxonomic affiliations.

The current findings are significant in the scope of recent concerns about diversity loss and decline of ecosystem stability (Danovaro et al., 2008). What maintains the ecological stability is the variety of functional roles that species play in a given ecosystem, not necessarily its taxonomical identities. In order to preserve the ecological stability, it is pivotal to identify the underlying environmental factors that drive the functional response of biota, once that the environmental factors governing the nematode functional distribution patterns are different from those driving their taxonomic composition (Boström et al., 2006; Conde et al., 2013). Our results demonstrated that biological traits introduce a new dimensionality in multivariate data that otherwise could not be detected using solely taxonomical information, thereby enhancing our knowledge on ecological gradients existing within an estuary.

4.2. Environmental drivers of the taxonomy and functional diversity indices

When taxonomical and functional indices were displayed in the multivariate space, its ordination reflected similar gradients to those identified by the nematode assemblage data. Similarly, both forms of oxygen were highly and significantly responsible for driving the spatial ordination of the diversity and functional metrics. The main gradient has discriminated sampling stations located next to the estuary mouth characterized by coarser grain size (sand) and higher concentrations of dissolved oxygen. This group of sites had the highest value of FRic, representing an amount of functional niche space filled by the community (Villéger et al., 2008). On the opposite end, muddy, silt&clay dominated habitats, richer in organic matter deposits and with less dissolved oxygen had a higher functional divergence (FDiv). FDiv reflects complementarity of niches among taxa and high FDiv might indicate low competition for the resources and hence lower niche overlap (Mason et al., 2005). FEve and FDis as well Pielou evenness were also negatively correlated with the FRic and levels of oxygen saturation. FEve indicates how well the species are distributed in their niche space, and FDis correspond to Rao's quadratic entropy and represents the average distance in the traits space to the centroid weighted by the species abundance. Hence, the higher FDis and FEve the better the taxa are distributed in the traits space, indicating lower niche overlap and better resource utilization. It appears that sampling stations located in the proximity of estuary mouth with coarse sediment grain size and richer in dissolved oxygen provide better conditions for more specialist type of traits (i.e. epistrate feeders, predators, k-strategy taxa), while increasing the interspecific competition for less abundant resources resulting in higher niche overlap. On the other hand, more generalist type of taxa (deposit feeders) dominates depositional areas of fine sediment and reduced oxygen concentrations, being responsible for lower trait diversity. However, these assemblages seem perfectly adapted to these conditions (as indicated by the high FDiv), being able to take advantage of the abundant organic matter deposits, resulting in more uniform traits distribution, smaller niche overlap and higher rates of resource utilization. Finally, the index of species richness appeared to be largely affected by the salinity gradient where the lowest species richness was associated with the most upstream-located sampling stations.

Predicting how species loss will affect the ecosystem function and stability represents a key issue in ecological studies (Barnes et al., 2018). The global concern about anthropogenic stress affecting marine environments and concomitant biodiversity loss has triggered investigations to explore complex diversity-function relationship, most of which revealed so far contrasting and non-linear patterns. While some authors found a clear positive relationship between diversity and function (Danovaro et al., 2008; Schratzberger et al., 2007), others found no consistent patterns (Baldrighi and Manini, 2015; Leduc et al., 2013; Pape et al., 2013). Such discrepancies are likely dependent on the type of

functional traits used in the analysis, high level of biodiversity and species redundancy (Hooper et al., 2005), but also the ecosystem under study (Snelgrove, 1999). We found a strong correlation between functional diversity (FRic) and taxonomic metrics (species richness, Simpson and Shannon diversity), but no correlation was found between taxonomic diversity and single MI or ITD indices. This is probably because changes in phylogenetic diversity do not need to be explicitly related to changes in functional diversity (Warwick and Clarke, 2001), and genera aggregation into higher functional entities might fail to consider within group variation (Jansen et al., 2018; Wright et al., 2006). For example, nematodes that belong to the same trophic group, but had different life history strategies exert a divergent effect on soil ecosystem processes (Postma-Blaauw et al., 2005). Most available studies on meiofauna diversity-function relationship rely on single trait indices (Baldrighi and Manini, 2015; Leduc et al., 2013; Pape et al., 2013). However, several papers on this topic have highlighted that using the combinations of traits (multi-trait indices) or some measure of ecosystem functioning (i.e. organic matter decomposition) offer a more realistic insight into the diversity-ecosystem relationship, resulting in clearer patterns between taxonomical and functional diversity (Danovaro et al., 2008; Gagic et al., 2015; Schratzberger et al., 2007; Stuart-Smith et al., 2013). Our results corroborate these observations, since when single functional indices (MI and ITD) were analysed separately there was no pattern in which taxonomic diversity would enhance functional diversity. However, when both traits were combined together and expressed as functional richness, a positive significant relationship with species richness and diversity indices was found. Functional richness describes how much of the functional niche space is filled by the existing species and represents a value of a convex hull volume in a two-dimensional space (each trait represents one axis) filled by a community (Cornwell et al., 2006; Laliberté and Legendre, 2010; Villéger et al., 2008). Combining both traits to define a convex hull volume predicts better the diversity measures than if each trait is used alone, indicating a certain complementarity between trophic diversity and life history strategies. Additionally, our results demonstrated that only these two traits had the capability to represent the array of basic functions performed by nematode assemblage and, in spite of the high biodiversity found in the Sado Estuary, species redundancy was relatively low. We also obtained significant correlations between functional dispersion and Simpson diversity and measures of evenness (Shannon entropy and Pielou evenness). Similar finding was also reported for the macrofauna communities in Yangtze River Estuary (Zhong et al., 2020). Functional dispersion measures the dispersion of species in a trait space, also accounting for the relative abundances of species. High correlation between functional dispersion and evenness indicates that nematode's traits distribution corresponds to its taxonomical analogues, and for example at sampling stations that host rare species, these species also possess extreme trait values. To provide for a better insight into diversity-ecosystem function relationship, future studies should carefully consider as many traits as possible to cover the wide array of functions performed by nematodes, and test these relationships across various environmental conditions. Nevertheless, some traits are hardly difficult to obtain or highly time-consuming to measure, and sometimes only few basic traits are available for analysis. In these cases, statistical tools that enables the calculation of functional diversity based on multiple available traits are likely to better capture diversity-function relationships than a single set of traits or indices (Mouchet et al., 2010; Petchey and Gaston, 2002). Furthermore, significant correlation obtained between functional dispersion and Simpson diversity demonstrate that functional dispersion can be another potentially informative metric to explore future diversity-function relationships.

5. Conclusions

Our results clearly demonstrate that nematode's functional response differs from the taxonomy-based approach in relation to an

environmental gradient. Moreover, the functional traits introduce a new dimensionality in a multivariate data that otherwise could not be detected using solely taxonomical information, thereby enhancing our knowledge on ecological gradients existing within an estuary. Additionally, the fact that both forms of dissolved oxygen were significantly correlated not only with the functional traits dataset, but also with the taxonomical and functional diversity indices, draw attention to the role that above sediment dissolved oxygen concentration exerts on nematode communities and their functional traits distribution patterns. The high complementarity of both functional indices (ITD and MI) in reflecting and complementing taxonomic diversity measures validates their combined usage for nematode-based ecological quality assessment.

In summary, our findings imply that an efficient insight into the nematode ecological response to an environmental gradient requires simultaneously looking at the assemblage response from both functional and taxonomy-based perspectives. This finding has important implications for the sustainable ecosystem management, as it demonstrates that only the integration of these two approaches will succeed in a more efficient assessment of the “good ecological status”. Moreover, an efficient long-term ecosystem management requires a good assessment of ecosystem stability, for which the functional roles of different genera showed to be a reliable proxy. These conclusions constitute an important argument for potential integration and implementation of benthic nematodes assessments into the future biomonitoring actions within

sustainable ecosystem management plans.

CRedit authorship contribution statement

Kasia Sroczynska: Conceptualization, Formal analysis, Writing - original draft. **Paula Chainho:** Writing - review & editing. **Soraia Vieira:** Methodology, Writing - review & editing. **Helena Adão:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1. Environmental parameters measured at each sampling station in Sado Estuary, Portugal.

Sampling station	salinity	temperature (°C)	pH	dissolved oxygen (%)	dissolved oxygen (mg L ⁻¹)	depth (m)	TOM (%)	TOM (g)	gravel (%)	sand (%)	fine sand (%)	silt&clay (%)
S1	0.75	16.94	7.85	68.60	6.60	3.06	0.82	0.04	17.74	69.21	8.34	4.70
S2	1.20	17.06	7.84	68.70	6.58	2.48	0.51	0.02	2.25	66.17	28.64	2.94
S3	3.59	17.43	7.78	75.70	7.09	1.14	10.28	0.49	0.09	0.52	1.52	97.87
S4	5.58	17.39	7.72	75.10	6.97	1.98	7.26	0.36	15.01	5.06	13.11	66.83
S5	5.53	17.47	7.74	75.40	6.98	2.74	0.88	0.04	16.68	54.84	26.79	1.68
S6	13.62	17.68	7.76	75.20	6.40	2.00	1.31	0.04	0.60	34.66	51.99	12.75
S7	13.46	17.64	7.24	86.00	2.61	4.19	7.49	0.35	0.70	8.63	13.57	77.09
S8	17.24	17.59	7.81	82.20	7.08	9.00	8.89	0.42	0.17	2.59	4.68	92.56
S9	17.14	17.63	7.49	45.40	3.27	5.65	10.79	0.43	0.00	0.07	0.14	99.79
S10	19.11	17.88	7.85	90.20	7.62	1.93	10.75	0.54	0.07	0.09	0.40	99.44
S11	17.46	16.78	7.82	94.70	8.52	0.14	10.85	0.31	0.06	0.49	0.40	99.05
S12	18.77	17.92	7.87	86.20	7.31	0.68	9.50	0.27	0.05	0.16	0.11	99.68
S13	20.36	17.58	7.89	91.30	7.76	0.09	9.28	0.44	0.11	0.13	0.09	99.67
S14	20.71	17.78	7.91	88.40	7.42	0.72	10.12	0.47	32.51	4.40	2.25	60.85
S15	21.40	17.55	7.90	86.10	7.23	2.14	8.71	0.40	31.93	6.47	5.93	55.67
S16	24.75	17.22	7.93	73.30	5.58	3.13	9.44	0.26	0.15	0.26	1.09	98.50
S17	23.54	17.69	7.95	88.80	7.35	1.09	5.43	0.20	19.95	11.09	7.99	60.96
S18	29.87	17.70	8.02	94.20	7.50	0.96	1.80	0.06	0.51	7.50	18.42	73.57
S19	27.78	17.54	8.00	89.80	7.26	2.35	8.60	0.40	39.69	1.70	0.85	57.76
S20	27.57	17.62	8.01	92.80	7.50	5.24	3.73	0.11	7.08	11.23	32.81	48.87
S21	31.73	17.17	8.07	92.80	7.38	7.00	2.25	0.07	19.61	23.35	38.40	18.64
S22	34.72	16.12	8.11	97.50	7.77	10.00	1.93	0.06	4.32	46.33	26.48	22.88
S23	35.10	15.65	8.15	97.10	7.79	4.00	1.28	0.04	5.60	51.72	27.07	15.61
S24	35.34	15.50	8.16	97.10	7.81	5.00	1.26	0.04	6.28	38.82	29.08	25.82
S25	30.19	17.67	8.06	94.00	7.47	1.72	10.02	0.25	0.00	0.32	0.66	99.02
S26	29.79	17.76	8.07	95.20	7.58	4.37	9.55	0.28	12.28	4.63	4.41	78.68
S27	31.12	17.36	8.08	94.50	7.51	1.73	5.38	0.16	24.49	6.45	4.15	64.91
S28	29.80	17.76	8.06	95.20	7.57	1.74	3.31	0.10	4.53	10.99	33.77	50.71
S29	30.75	17.23	8.01	94.00	7.51	4.00	9.63	0.45	1.37	2.26	4.74	91.63
S30	31.00	17.21	8.05	91.30	7.29	4.00	0.39	0.02	25.31	34.73	33.79	6.17
S31	29.47	16.82	7.96	87.20	7.08	1.00	8.98	0.26	1.44	0.82	0.87	96.88
S32	30.24	16.60	7.98	88.20	7.16	0.90	8.89	0.27	1.57	0.36	0.28	97.79
S33	29.77	16.53	7.97	88.60	7.22	0.40	11.13	0.49	0.09	0.11	0.10	99.70
S34	29.47	16.90	7.95	86.10	6.97	1.00	9.64	0.28	50.83	4.96	2.75	41.46
S35	30.54	17.28	8.03	92.90	7.43	0.90	2.60	0.06	5.49	16.98	29.66	47.87

Appendix 2. Density (ind. 10⁻²), trophic group and c-p score of the 20 most abundant nematode genera at each sampling station in Sado Estuary, Portugal.

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