Evaluating environmental drivers of spatial variability in free-living nematode assemblages along the Portuguese margin

Lidia Lins[1,2], Frederik Leliaert[1], Torben Riehl[3], Sofia Pinto Ramalho[1,4], Eliana Alfaro Cordova[5], André Morgado Esteves[2], Ann Vanreusel[1]

[1]Marine Biology research group, Ghent University, Krijgslaan 281 S8, 9000 Ghent, Belgium, lidia.linspereira@ugent.be, Tel:+32 (0)9 264 8531
[2]Meiofauna Laboratory, Federal University of Pernambuco, Av. Prof Moraes Rego 1235, 50670-901, Cidade Universitária, Brazil
[3]CeNak, Center of Natural History, University of Hamburg - Zoological Museum, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany
[4]Departamento de Biologia & CESAM, Universidade de Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal
[5]Prodelphinus, Jose Galvez 780-E, Lima 18, Peru

Correspondence to: Lidia Lins (lidia.linspereira@ugent.be)

Abstract

Understanding processes responsible for shaping biodiversity patterns on continental margins is an important requirement for comprehending anthropogenic impacts in these environments and further management of biodiversity. Continental margins perform crucial functions linked to key ecological processes which are mainly structured by surface primary productivity and particulate organic matter flux to the seafloor, but also by heterogeneity in seafloor characteristics. However, to what extent these processes control local and regional biodiversity remains unclear. In this study, two isobathic parallel transects located at the shelf break (300–400 m) and upper slope (1000 m) of the Western Iberian margin were used to test how food input and sediment heterogeneity affect nematode diversity independently from spatial factors. We also examined the potential role of connectedness between both depth transects through molecular phylogenetic analyses. Local and regional generic diversity and turnover were investigated at four levels: within a core, within a station, between stations from the same depth transect, and between transects. High variability in food availability and high sediment heterogeneity at the shelf-break transect were directly linked to high diversity within stations, and higher variation in community structure across stations compared to the upper slope transect. Contrastingly, environmental factors (food availability and sediment) did not vary significantly between stations located at the upper slope, and this lack of differences were also reflected in a low community turnover between these deeper stations. Finally, differences in nematode communities between both transects were more pronounced than differences within each of the isobathic transects, but these shifts were paralleled by the before-mentioned environmental shifts. These results suggest that changes in community structure are mainly dictated by environmental factors rather than spatial differences at the Western Iberian Margin. Furthermore, phylogenetic relationships revealed no evidence for depth-endemic lineages, indicating regular species interchanges across different depths.

1. Introduction

The link between biodiversity (i.e. species diversity) and ecological processes (e.g. carbon flow, surface productivity) has created a heightened interest in ecological research after large-scale human impacts were deemed responsible for declining species numbers and alterations of ecosystem properties (Loreau et al., 2001). Stretching between the coastal zone and the abyssal plains of the deep sea, continental margins (100–4000 m) encompass the largest habitat diversity in the marine environment (Levin and Dayton, 2009; Ramirez-Llodra et al., 2010). They harbour a high biodiversity, and are responsible for 90 % of the new biological productivity in oceans and seas, providing valuable food and energy resources for the marine fauna (Salgueiro et al., 2014).

It is generally accepted that principal biological oceanographic processes, such as carbon burial and nutrient cycling, remain concentrated within continental margins (Levin and Dayton, 2009). Yet, the biodiversity of continental margins is under severe threat by increasing commercial exploitation, ranging from fisheries, to gas, oil, and mineral extraction (Levin and Dayton, 2009; Puig et al., 2012). The direct impact of these unabated commercial activities on benthic environment and populations varies greatly, from pervasive sediment erosion, transportation and deposition, to the large-scale alteration of community composition (Puig et al., 2012). Therefore, continental margins comprise key locations to study the effects of environmental alterations on benthic biodiversity.

Understanding the processes that shape biodiversity patterns on continental margins is an important prerequisite for comprehending and managing anthropogenic impacts in these environments. Sea-surface processes have an important effect on the benthic fauna because part of the primary production is exported from overlying waters to the deep-sea floor, mostly in the form of phytodetritus, where it serves as food source to benthic communities (Billett et al., 1983; Lins et al., 2015; Serpetti et al., 2013; Wei et al., 2010). Particulate organic carbon input in the deep sea has been regarded as one of the main factors shaping benthic community structure and functioning (Rex, 1981). Phytodetritus creates patchiness, enhancing habitat heterogeneity, and consequently promotes species coexistence (Cardinale et al., 2000). In addition, depth indirectly plays a role in structuring benthic communities, since organic matter flux is negatively related to depth, and deeper regions will consistently receive less input of labile organic matter compared to shallower regions (Danovaro et al., 2010; Garcia and Thomsen, 2008; Lutz et al., 2007; Ramalho et al., 2014). As a consequence of this decline in food availability, decreases in abundance and biomass associated with an increase in depth on the continental slopes have been observed for all benthic size classes (mega-, macro-, and meiofauna) (Flach et al., 2002; Muthumbi et al., 2011; Rex et al., 2005; Rowe et al., 2008; Thiel, 1978).

Food availability, as well as biological factors (predation, competition, dispersal), are assumed to drive small-scale (1–10 m²) patterns of benthic communities (Gage, 1997) promoting local diversity (Levin et al., 2001). Community differences

2

over large spatial scales (100–1000 m²; beta diversity) within continental margins have been observed both along bathymetric gradients as well as between different sites at similar depths. This indicates that beta diversity is not singularly depth-dependent (Danovaro et al., 2013; Easton and Thistle, 2016; Havermans et al., 2013; Leduc et al., 2012a). Physical factors, including near-bottom currents, sediment grain-size heterogeneity, boundary constraints, human activities, and topography are also considered of particular importance for beta diversity (Levin et al., 2001). They shape biodiversity as they may reduce the effect of a dominant species through the redistribution of resources among inferior and superior competitors (Stachowicz et al., 2007), and in this way increasing species diversity.

Moreover, population dynamics and dispersal (Derycke et al., 2013; Gage, 1997; Rex et al., 2005) have been shown to affect the structuring of benthic fauna at different spatial scales. Most benthic species have restricted active dispersal potential, although passive dispersal may be facilitated through ocean currents (Etter and Bower, 2015; Gallucci et al., 2008; Lins et al., 2014; Ullberg and Olafsson, 2003). The lack of a pelagic larval stage in free-living nematodes, the focus group of this study, could therefore be viewed as a disadvantage to dispersal. Nevertheless, this abundant and omnipresent group of benthic metazoans is found at all depths and in all deep-sea habitats (Giere, 2009; Vincx et al., 1994). Nematodes belonging to the meiofauna (< 1 mm) exhibit high species richness and are one of the few taxa in which true cosmopolitan species may exist (Bik et al., 2010; Zeppilli et al., 2011). Some species are able to actively swim, following chemical cues, but more importantly, nematodes may be passively transported via water currents following resuspension from disturbance events (Jensen, 1981; Schratzberger et al., 2004). Molecular studies have indicated that different nematode taxa in diverse habitats exhibit population connectivity across a wide range, with some species showing subtle but significant genetic structuring at a small spatial scale, and other species exhibiting no differentiation along large distances (> 500 km). These findings confirm a high dispersal potential and low endemism for at least some species (Derycke et al., 2013, 2005). Nematodes therefore, hold ideal life traits when seeking to understand dispersal, coexistence, and benthic-pelagic coupling in the deep sea.

Depth-related factors are thought to inhibit across-depth gene flow and thus to promote speciation in some taxa. This depth-range limitation provides another explanation for why the bathyal holds such a high biodiversity (Rex and Etter, 2010). However, while empirical data for macrofaunal molluscs, crustaceans, as well as octocorals has been found supporting this depth-differentiation hypothesis (France and Kocher, 1996; Jennings et al., 2013; Quattrini et al., 2015), it may not apply to nematodes, where repeated and regular interchanges between depths were observed (Bik et al., 2010).

Most previous research on nematode diversity has concentrated either on bathymetric differences (Danovaro et al., 2013; Leduc et al., 2012a; Muthumbi et al., 2011), or on geographical transects and macro-habitat heterogeneity (Baldrighi et al., 2014; Lambshead et al., 2000; Van Gaever et al., 2009). No studies so far combined bathymetric with geographic analyses at a regional scale, which is crucial to understand patterns of biodiversity. Furthermore, the transition from the shelf to the slope remains a largely understudied area (Muthumbi et al., 2011; Vanreusel et al., 1992), while major environmental shifts are observed here. Through the analysis of two isobathic transects of about 20 km length, and separated by 30 km and 600 m water depth, we tested the effect of environmental (food availability and sediment heterogeneity) and spatial (depth and geographical distance) variables on nematode diversity. In this way, drivers for turnover in nematode taxonomic composition

were analysed at four spatial scales: within a core, within stations, between stations from the same depth, and between two depth transects. As food input and sediment heterogeneity are expected to vary more with depth than along regional isobathic transects, we expected a higher turnover in community composition between depths than within isobathic transects. To evaluate possible depth-mediated differentiation, genus turnover and phylogenetic relationships through DNA sequence clustering between the two isobathic transects was investigated based on 18S rDNA sequence data of selected nematode taxa. The following hypotheses were tested: (H1) Higher patchiness of food resources deposited at the seafloor results in a higher local diversity; (H2) Increased sediment heterogeneity results in a higher beta diversity; (H3) Beta diversity between different bathymetric transects is higher than beta diversity across similar depths; (H4) Clades/Taxa are shared between shelf break and slope areas.

2. Material and methods

2.1. Sampling and study area

The Western Iberian Margin (WIM) is characterized by a narrow shelf and steep slope (Garcia and Thomsen, 2008; Nolasco et al., 2013; Relvas et al., 2007). Primary production in this area increases in May–June and constitutes a significant proportion of the yearly production, reaching values higher than 90 gCm²y^{-1} (Salgueiro et al., 2014). The WIM exhibits seasonal upwelling with filaments that can penetrate more than 200 km into the open ocean, influencing not only vertical transport but also horizontal particle transport from near shore towards the open ocean (Crespo et al., 2011; Figueiras et al., 2002; Relvas et al., 2007; Salgueiro et al., 2014, 2010). The high particle transport observed at the WIM occurs mainly due to the great bottom dynamics in the area. This region possesses an equatorward current flow generated by thermohaline structures of water masses and wind-forcing, eddy interactions with the alongshore circulation and buoyant plumes (Relvas et al., 2007). These features, together with shelf and coastal currents, upwelling filaments, and fronts, impact the subsurface circulation, internal waves, and consequently the transport of sinking particulate organic matter to the seabed (Alvarez-Salgado et al., 1997; Relvas et al., 2007).

During the RV Belgica B2013/17 (10.06.2013–18.06.2013) and B2014/15 (02.06.2014–10.06.2014) cruises to the WIM, sediment samples for nematode and environmental analyses were taken at the slope off the southwest coast of Portugal (Fig. 1). The study area comprised two main transects roughly parallel to the isobaths. The first transect was 23 km long, situated 294–445 m deep (further referred to as shallow transect), just beyond the shelf break; the second transect was located at the upper-slope, 19 km long, and at a water depth of 900–1006 m (named deep transect). The 'shallow' area included six stations while the 'deep' area comprised four stations (Table 1). Sampling was performed using a Multicorer (MUC) equipped with four Plexiglas tubes yielding samples with a virtually undisturbed sediment surface (inner core diameter 9.8 cm).

2.2. Sediment analyses

Three to four replicated samples for granulometric and geochemical analyses (1 g of sediment) from the first sediment layer (0–1 cm) were frozen at -80 °C. Grain-size distribution was measured with a Malvern Mastersizer 2000 (0.02–2000 µm

4

size range) and divided into five categories, from silt-clay to coarse sand fractions. Sediment particle-size diversity (SED) was calculated from the percent dry weight of the five size classes mentioned above using the Shanon-Wiener diversity index (Etter and Grassle, 1992; Leduc et al., 2012b). Total sedimentary organic carbon (% TOC) and nitrogen (% TN) were determined with a Carlo Erba elemental analyser on freeze-dried and homogenized samples after acidification with 1 % HCl to eliminate carbonates. Total organic matter (% TOM) content was determined after combustion of the sediment samples at 550 °C.

Chlorophyll a (Chla), chlorophyll degradation products, and carotenoids in the sediment were measured with a Gibson fluorescence detector (Wright and Jeffrey, 1997) after lyophilisation, homogenization, and extraction in 90 % acetone, and separation of the samples via reverse-phase HPLC (High-Performance Liquid Chromatography). Chloroplastic pigment equivalents (CPE: Chla + phaeopigments) were used as a proxy for surface-derived primary productivity at the seafloor.

2.3. Nematode sample processing for community analyses

At each station, three to four replicate samples of the 0–1 cm layer were used for nematode analysis. Samples were fixed on board with seawater buffered 4 % formalin. Sediment was washed over 1000 µm and 32 µm sieves. The fraction retained on a 32 µm sieve was centrifuged three times using LUDOX HS40 Dupont (specific gravity 1.19) as flotation medium and then stained with Rose Bengal. In each sample, 140 nematode individuals (whenever enough present) were randomly picked out and gradually transferred to glycerine (De Grisse, 1969), mounted on glass slides and identified to genus level using relevant literature (Vanaverbeke et al., 2015; Warwick et al., 1998).

Functional diversity (relative abundance of each trophic type) of nematodes was calculated using individuals trophic levels according to Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A), and predators (2B), complementing the 2B group with the notion of 'scavengers' (Jensen, 1987). Trophic diversity (TD) was calculated using the index proposed by Heip et al. (1985):

$$TD = \frac{1}{\sum_{i=1}^{4} qi^2},$$

where q_i is the relative abundance of type i. Taxonomic diversity was measured using Shannon-Wiener diversity (H'), expected nematode genera (EG (80)) and Pielou's evenness (J'). Each replicate (core) value was used as a measurement of alpha diversity, while differences within stations, between stations and between transects were utilized to measure beta diversity.

2.4. Data analysis

Trends in environmental variables (% TOC, % TN, % TOM, Chla, CPE, carotenes, depth, sediment grain size and SED) and univariate nematode variables (H', J', EG (80), and TD) were investigated by means of Spearman rank correlations and Draftsman plots (Anderson et al., 2007) in R (R Core Team, 2013).

The nematode community data based on relative abundance of genera were analysed based on Bray-Curtis similarities (and Euclidean distances for the univariate data) by means of non-parametric multivariate ANOVA (PERMANOVA; Anderson et al., 2007) to assess differences between 'deep' (slope) and 'shallow' (shelf-break) areas (2-factor nested design). The 2-factor model included 'depth' as a fixed factor and 'station' as a random factor nested in 'depth'. Due to the use of an

unbalanced design, the type I of sum of squares was chosen for the PERMANOVA analysis to make sure all possible re-arrangements of samples are equally likely (Anderson et al., 2007). Subsequent pairwise pseudo *t*-tests were performed between all pairs of levels to determine where differences between each combination were found. Additionally, PERMDISP routines were used to test for homogeneity of multivariate dispersions between stations. PERMDISP results were not significant, indicating location differences through equally dispersed distances to centroids. SIMPER routines were executed based on Bray-Curtis similarity, with a cut-off of 90 % for low contributions. Dissimilarities within and between stations were compared with distances between geographical areas (km) and between depth differences (m).

The multivariate environmental data was first normalized (subtracted mean divided by standard deviation) and resemblance matrices were calculated based on Euclidean distances. Subsequently, PERMANOVA tests were performed using the same design as described for the multivariate nematode community data. DistLM (distance-based linear model) routines were performed to analyse and model the relationship between nematode genus community and environmental variables with correlations lower than 0.9 (Chla, carotenes, CPE, % TN, silt-clay, very fine sand, medium sand, and coarse sand). Highly correlated variables (% TOC, % TOM, fine sand, and depth) were first transformed to cosine and if high correlations persisted they were excluded from the DistLM analysis. The DistLM model was built using a step-wise selection procedure and adjusted R^2 as a selection criterion. Euclidean distance was used as a resemblance measure for DistLM procedures and the results were displayed in dbRDA (distance-based redundancy analysis) plots.

2.5. Molecular phylogenetic analyses of nematodes

One sample (replicate) from each of the 'shallow' stations S4 and S2 and one from the 'deep' station D4 were preserved in DESS (Yoder et al., 2006) and used for molecular analyses. The first centimetre (0–1 cm) of each core was washed with LUDOX HS40 Dupont, following the same protocol as for the community analysis (see above). One hundred nematodes were randomly picked out per sample under a stereomicroscope (50x magnification). Each individual was rinsed in sterile water, transferred to a microscope slide containing sterile water, and digitally photographed as morphological reference with a compound microscope Leica DMR and Leica LAS 3.3 imaging software. DNA extraction followed Derycke et al. (2005) using the entire specimens.

PCR amplification of the nuclear small subunit (SSU or 18S) rDNA was conducted using the primers G18S4 (5'-GCTTGTCTCAAAGATTAAGCC-3') and 22R (5'-GCCTGCTGCCTTCCTTGGA-3') (Blaxter et al., 1998). All PCR reactions were conducted using an EXT PCR Kit, with a final reaction volume of 25 µl. Each reaction contained 2 µl of template solution containing nematode genomic DNA, 15.125 µl PCR grade water, 0.125 µl of each primer (25 mM), 2.5 µl 10x of PCR buffer, 2 µl of $MgCl_2$, 2.5 µl Loading dye, 0.5 µl dNTP 10mM and 0.125 µl DNA TopTaq polymerase. PCR amplifications were conducted for 39 cycles, each consisting of a 30s denaturation at 94 °C, 30 s annealing at 56 °C, and 30 s extension at 72 °C, with an initial denaturation step of 5 min at 94 °C and a final extension step of 10 min at 72 °C.

Successful PCR reactions were identified using agarose gels stained with ethidium bromide and were sequenced with both forward and reverse primers by Macrogen Europe (The Netherlands) with the fluorescent dye terminator Sanger

sequencing method. The resulting reads were assembled using Mega 6.0 (Tamura et al., 2013). Sequences were checked for contamination using the BLAST algorithm on GenBank (Benson et al., 2008). The sequences that showed contamination or were of low quality (high amount of ambiguous nucleotides) were removed from the alignment. Nematode contig sequences (consensus of forward and reverse sequences) generated during this study were aligned using the MAFFT multiple sequence alignment algorithm (Katoh et al., 2009) as implemented in Geneious 9.0 (Kearse et al., 2012) at default settings (the alignment algorithm was automatically determined; scoring matrix was 200PAM / k=2; gap-opening penalty was 1.53 and the offset value was 0.123).

GenBank sequences for the most representative genera in the samples (all of the nematode class Chromadorea) were included from GenBank (Benson et al., 2008) (whenever available) to compare differences in genetic/phylogenetic diversity between different depths and locations. Sequences from Meldal et al. (2007) and from Bik et al. (2010) were used to compare generic diversity and diversity within the genus *Halalaimus*, respectively, between different habitats.

For both datasets, Modeltest 2.1 (Posada and Crandall, 1998) and jModeltest (Posada, 2008) were used to determine that the best suitable model for maximum likelihood analyses of the nuclear data was according to the Akaike Information Criterion (AIC) (Akaike, 1981) GTR+I+G.

Reconstruction of 18S relationships was conducted using Maximum Likelihood. The analyses were performed by means of Randomized Axelerated Maximum Likelihood (RAxML) (Stamatakis, 2006) in raxmlGUI (Silvestro and Michalak, 2012) using the fast Likelihood search with 1000 replicates to calculate Bootstrap support values.

For the *Halalaimus* dataset, Bayesian inference was additionally applied in MrBayes (Ronquist and Huelsenbeck, 2003) to supplement topological inferences. Analyses were run for 5 000 000 generations using 6 MCMC chains. From all runs the first 25 % of sampled trees were discarded as burn-in. Consensus trees were used for illustration here and were ordered and annotated in FigTree and Geneious tree viewer and colorized in Adobe Illustrator. In the supplement tree, line thickness indicates strength of bootstrap support.

3. Results

3.1. Environmental parameters

Biogeochemical and granulometric properties of the sediment are shown in Figure 2. Nested PERMANOVA results for sediment particle-size diversity (SED) revealed significantly lower values ($p < 0.05$) at the 'deep' transect. Pairwise comparisons for SED showed significant differences for the pairs [D2, D3] at the 'deep' transect and for all the pairs at the 'shallow' transect, except for [S1, S4], [S2, S614], and [S2, S613]. Sediment composition at the 'deep' stations was mainly composed of silt-clay fractions (81–89 %), while at the 'shallow' stations sediment was more heterogeneous. At the shelf-break, fine sand (25–42 %) dominated, except for S7, where medium sand showed a higher proportion (30 %). Nested PERMANOVA results showed significant differences in sediment composition between depth transects and among stations within the same transect ($p < 0.05$) (Table S1). Pairwise comparisons between stations showed higher variability in sediment composition for the 'shallow' stations, where the pairs of stations [S7, S2], [S2, S614], [S2, S613], and [S614, S613] showed

similar sediment characteristics (Table S1). Pairwise comparison for 'deep' stations only showed differences between D2 and D3. Within station comparison showed low variability (< 25 % deviation from the mean values) in silt-clay and very fine sand for most stations both shallow and deep (Fig. 2). Fine, medium, and coarse sand variability within each station was higher when compared to silt-clay and very fine sand (Fig. 2). Significant higher values (nested PERMANOVA, $p < 0.05$) of % TOM

5   (Table S2), % TOC (Table S3), and % TN (Table S4) were observed at the deeper transect. Additionally, 'deep' stations were not significantly different from each other ($p > 0.05$) for % TOM, % TOC and % TN, while 'shallow' stations exhibited significant differences between pairs of stations for % TOM (significantly different pairs: [S1, S4], [S1, S7], [S4, S7], [S4, S2], [S4, S614], and [S4, S613]), % TOC (significantly different pairs: [S1, S4], [S4, S2], [S4, S613], and [S614, S613]), and % TN (significantly different pairs: [S1, S4], [S4, S7], and [S4, S2]). No strong variability (< 25 % deviation from the mean

10   values) was observed within stations for these three variables (Fig. 2). Chla (0–0.17 µg g^{-1}), carotenes (0–0.72 µg g^{-1}) and CPE (0.01–1.79 µg g^{-1}) values were generally low. Chla showed no significant differences between depth transects ($p > 0.05$) and only the pairs [S1, S7] and [S1, S614] were significantly different from each other (Table S5). In addition, Chla showed high variability (> 25 % deviation from the mean values) at the 'shallow' stations, especially at S4 (Fig 2). Carotenes and CPE also possessed high variability (> 25 % deviation from the mean values) at the shelf-break stations, and revealed significant

15   differences between depths and among pairs of stations ($p < 0.05$). For carotenes, the pairs of stations [S1, S4], [S1, S7], and [S1, S614] were significantly different from each other (Table S6), while for CPE only the pair [S1, S613] was significantly different (Table S7). Moreover, carotenes were completely absent at the 'deep' stations (Fig. 2).

3.2. Nematode community structure

The most abundant nematode genera (≥ 4 %) per station are visualized in Fig. 3. The genera *Acantholaimus* and

20   *Halalaimus* dominated at all 'deep' stations (7.6–11.3 % and 7.2–11.7 %, respectively), whereas the 'shallow' stations showed high variability in the most abundant genera. Among the 155 identified genera, 62 were restricted to the 'shallow' areas, and 19 genera were only found in the 'deep' stations. Most of the genera showed low occurrence, with 87 genera found at relative abundances < 1 %. Evenness (J') was not different between the two bathymetric areas and only revealed pairwise differences between D2 and D4 (Table S8). Shannon-Wiener (H') diversity at genus level varied from 3.18 (S1) to 3.74 (S613) at the

25   'shallow' stations and from 3.17 (D3) to 3.43 (D1) at the 'deep' transect (Fig. 4). In this sense, Shannon-Wiener (H') diversity was significantly higher at 'shallow' stations (nested PERMANOVA, $p < 0.05$), and the highest diversity was observed at S613 (Fig 4, Table S9). Pairwise comparisons revealed significant differences between the pairs [S1, S7], [S7, S2], and [S2, S614]. Besides having the highest diversity, S613 also revealed the highest replicate variability (3.52 ± 0.19). Expected number of genera (EG(80)) varied from 27 (S2) to 34 (S4) at the 'shallow' stations and from 25 (D2) to 31 (D1) at the 'deep' stations

30   (Fig 4). Nested PERMANOVA results revealed significant higher values at the 'shallow' stations when compared to the 'deep' stations ($p < 0.05$) for EG (80) (Table S10). Pairwise comparisons showed significant differences between the pairs [S1, S7], [S1, S614], [S7, S2], and [S2, S614]. Total nematode density was significantly higher at the 'shallow' stations when compared

to the 'deep' stations (p < 0.05), but pairwise comparisons revealed no significant differences between pairs of stations (Fig. 4).

Dissimilarities between deployments within stations varied from 53–60 % (S1), 38–57 % (S2), 45–57 % (S4), 45–57 % (S613), 41–53 % (S614), and 42–58 % (S7) for the shallow stations, and 39–60 % (D1), 39–51 % (D2), 36–47 % (D3), and 44–64 % (D4) for the deep stations, thus revealing similar within-station variation for both transects. Moreover, dissimilarity values between stations increased with increasing depth and geographical distance (Fig 5). SIMPER analysis revealed that the genera *Acantholaimus, Microlaimus*, *Richtersia,* and *Halalaimus* were mainly responsible for the average dissimilarity (63.99 %) between the two depths. The first genus was mainly found at 'deep' areas, whereas *Microlaimus* and *Richtersia* had higher densities at 'shallow' stations. The genus *Halalaimus* showed similar average densities in both transects, but higher density fluctuations at the 'shallow' stations. Nested PERMANOVA results showed significant differences between transects and among stations (p < 0.05) (Table S11). Pairwise comparisons revealed no significant differences between 'deep' stations, while the pairs of 'shallow' stations [S1, S7] and [S7, S2] possessed significant differences (p < 0.05) in nematode genera composition.

Trophic diversity revealed significantly higher values at the shallow stations (p < 0.05) but no significant pairwise difference was observed for the shallow or deep stations (p > 0.05) (Fig. 4). Nested PERMANOVA results for relative abundance of trophic groups displayed significant differences between depths, but not among stations from the same depth (p > 0.05) (Table S12). Average similarity between 'deep' and 'shallow' areas was 80 %. SIMPER analyses revealed that differences between depths were mainly due to the higher relative abundance of selective deposit feeders (1A) at deeper stations. The shallow stations exhibited higher abundance of epistratum feeders (2A) and predators/scavengers (2B).

3.3. Correlation between nematode community structure and environmental variables

The correlation between univariate diversity values (H', J, EG (80), and TD) and environmental variables (% TN, % TOC, Chla, carotenes, CPE, sediment grain size, and SED) are shown in Table S13. Evenness (J') was not correlated to any environmental factor. Diversity (H') was negatively correlated to silt-clay, but positively correlated to very fine-medium sand, SED, and total carbon and nitrogen. The EG (80) was negatively correlated to % TN, % TOC, and silt-clay, and positively correlated to CPE (Fig. 6), very fine sand, fine sand, medium sand, and SED (Fig. 6). Trophic diversity (TD) was negatively correlated to % TN and silt-clay, and positively correlated to Chla, CPE (Fig. 6), very fine sand, fine sand, and SED (Fig. 6).

DistLM analyses based on twelve environmental variables explained 33 % of the total nematode diversity. Silt-clay accounted for 23 % of the total variation, being responsible for most differences found between 'shallow' and 'deep' stations (Fig. 7A). The other variables did not contribute significantly to the model and/or added < 5 % in explaining the total variation. When only 'shallow' stations were included in the model, the significant environmental variables explained 23 % of the total variation (Fig. 7B). Coarse sand was the main factor accounting for variation between 'shallow' stations (13 %). This sediment fraction showed highly fluctuating values (0.02–14.88 %) between stations of the 'shallow' areas.

3.4. Nematode molecular phylogenetic analyses

From the 300 vouchered nematodes, the success rate of sequencing was only 30 %. For 199 specimens no PCR product was detected or sequences were of low quality. Phylogenetic analyses showed that the 101 sequenced nematodes belong to seven different orders of free-living marine nematodes (Table S14). The highest genetic diversity was reported for the order Enoplida, with 25 different 18S sequences, followed by the order Plectida (19 different 18S sequences) and Desmodorida (18 different 18S sequences). The Maximum Likelihood (ML) phylogeny inferred from 18S sequences is shown in Fig. S1.

In general, the backbone of the Chromadorea phylogeny was poorly supported, leading to several paraphyletic or polyphyletic orders and some families, such as Plectida, Desmodorida, and Oxystominidae. Well supported were the orders Tylenchida (bootstrap support (bs) = 99), Monhysterida (bs = 100), Dorylaimida (bs = 100), Monochida (bs = 100), and Triblonchida (bs = 82). Desmodorida is polyphyletic in our analysis with the family Microlaimidae forming a well-supported clade (bs = 100). The orders Chromadorida and Enoplida represent monophyletic but extremely weakly supported groups (bs = 5 and 41 respectively), while the orders Trefusiida and Triplonchida appeared nested within Enoplida. However, resolving the phylogenetic ties within Chromadorea was not within the scope of this article. What the consensus shows is that the 18S phylogeny supported the broad taxonomic representation of nematodes in the samples and furthermore indicated neither geographic nor depth clustering between 'deep' and 'shallow' taxa at any level of the tree topology (Fig. S1). This was moreover demonstrated within the best-represented and monophyletic (Fig. S1) genus in the dataset, *Halalaimus* (15 individuals, complemented with 42 GenBank sequences from different depths and locations globally distributed). Here, the new sequences showed no clustering related to depth or geography was observed but instead they seem randomly scattered between samples from different depths and regions (Fig. 8).

4. Discussion

Taxonomic and trophic diversity per station and dissimilarity in genus composition between stations along the Western Iberian Margin (WIM) were higher at the shelf break ('shallow' stations) when compared to the upper slope ('deep' stations). The upper slope nematode communities were in general dominated by the same genera (*Halalaimus* and *Acantholaimus*), while at the shelf break not only a higher diversity was present per station, but also the genus composition clearly differed between stations, resulting in a higher turnover. This higher station diversity and along transect turnover at the shallower stations coincided with a higher amount and patchiness in food supply, as well as a higher sediment heterogeneity within and between stations. However, the largest community differences and therefore highest taxonomic turnover in nematode genera was present between both depth transects, while the high number of shared genera between transects and the intermingled pattern of genetic clustering observed for *Halalaimus* suggested that depth as such did not restrict the distribution of nematodes.

4.1. Sediment heterogeneity in combination with increased amount and patchiness of food resources contributes to a higher local diversity

In this study, local nematode diversity was significantly correlated with food availability. Moreover, the higher amount and variability of 'labile' food resources (Chla, CPE, and carotenes) within the 'shallow' stations when compared to the 'deep' stations (Fig. 2), was associated with a higher diversity in terms of trophic group and genus composition. The interactions between coastline features and wind forcing already reported for the WIM, certainly affect the export flux, and consequently the resource distribution on the seabed. For this region, the strong surface dynamics may explain the high patchiness of food input and habitat heterogeneity observed here, and consequently the high nematode local diversity (alpha and beta within station) exhibited at the shallow stations (Cardinale et al., 2000; Crespo et al., 2011; Tokeshi, 1999). Concurrently, a different pattern was observed for the deeper transect, where the lower amount and variability of food resources were associated with a decline in nematode diversity. A similar relationship between decreasing availability of 'labile' organic matter associated with a decrease in diversity was also observed at similar depths in other slope environments (Danovaro et al., 2013; Leduc et al., 2012a; Netto et al., 2005), and may thus represent a general pattern at continental margins.

The generally low food input to deep-sea sediments might favour organisms adapted to thrive in this food-deprived environment. In this study, the assumed deposit-feeding behaviour of *Halalaimus* may explain its success in both transects and reflect its general high fitness in the deep sea (Fig. 3), because deposit-feeding can be interpreted as an adaptation to the low availability and mainly refractory nature of food input (Amaro et al., 2009). Taxa with this feeding mode are often dominant in deep-sea soft sediments and may replace functions otherwise performed by more specialist genera (Amaro et al., 2009; McClain and Schlacher, 2015). The high abundances of the, as epistratum feeder classified, genus *Acantholaimus* (based on buccal morphology by Wieser (1953)) at the deeper transect was also expected (Fig. 3), since *Acantholaimus* is known as a typical genus for the deep sea (Miljutin and Miljutina, 2016; Muthumbi et al., 2011; Soetaert and Heip, 1995). It is indeed widely observed that *Acantholaimus* increases in relative abundance from the shelf break towards abyssal plains, while it dominates nematode communities at all greater water depths (Vanreusel et al., 2010). Until present, 51 species of *Acantholaimus* were described (Miljutin and Miljutina, 2016), while a large number of different species have been found to coexist (Muthumbi et al., 1997). By possessing a great variation in its mouth parts, *Acantholaimus* species coexistence indicates a possible food resource partitioning, rather than a general deposit-feeding behaviour as assumed for most deep-sea nematodes (Muthumbi et al., 2011). In this regard, the lower generic and trophic diversity observed in the deeper transect is possibly compensated by a higher species diversity and an associated flexible feeding strategy within the dominant taxa, such as *Acantholaimus*. The resource partitioning theory (Schoener, 1974) seems a plausible explanation for the success of *Acantholaimus* with high inter-specific variation concerning feeding structures.

However, the lower local diversity at the slope stations compared to the shelf break is not only explained by the lower and less patchy food input. Sediment heterogeneity (SED) was also positively correlated with the expected number of genera and the trophic diversity at a local scale, exhibiting even stronger correlation results than the one between nematode diversity

11

and CPE (Fig 6). The lower trophic diversity (TD) and higher relative abundances of deposit feeders in the deep stations already mentioned above, were potentially caused by a combination between low food input and comparatively higher sediment stability for this transect, reflected by the finer sediment composition in relation to the shallow stations (Fig. 2). This decline in TD with depth, positively associated with a decline in SED, was not observed in other studies along the slope, although the SED and TD values observed here were much higher than already reported before for other areas, and might reflect different environmental conditions at the WIM (Danovaro et al., 2013; Leduc et al., 2012b; Pape et al., 2013).

The higher trophic diversity at the shallow stations suggests a higher niche differentiation within this transect, reflecting the possible transient stage of this habitat. Moreover, higher prominence of opportunistic species and fast colonizers, such as *Microlaimus*, was observed at the shallower WIM stations (Fig. 3). The high abundance of opportunists has been reported for several bathyal areas in association with disturbance events (Muthumbi et al., 2011; Pape et al., 2013; Raes et al., 2010), pointing to potentially ongoing recolonization processes following the disturbances (Lee et al., 2001). The observed high densities of *Microlaimus*, which is considered both tolerant to disturbance and an early colonizer (Lee et al., 2001; Moreno et al., 2008; Raes et al., 2010), are in accordance with the assumed hydrodynamic regimes at the shallower stations and possible anthropogenic disturbance effects, for instance from fisheries, which further influence the bottom dynamics there (ICES, 2008; Quaresma et al., 2007; Relvas et al., 2007). Disturbance effects via either bedload movement or erosion and sedimentation of suspended load alters not only particle size, but also organic content. Thus, sediment heterogeneity in combination with increased patchiness of food resources deposited at the seafloor observed here were associated with a higher local generic and trophic diversity at the shelf break compared to deeper areas. Disturbance has been suggested before as a strong driver for diversity in the deep sea, with disturbances ranging from small-scale bioturbation traces, to intermediate-scale phytodetritus falls, or large scale currents (Levin and Dayton, 2009). In our study, similar disturbances resulting in sediment heterogeneity and food patchiness at the shelf break, are used to explain the higher diversity compared to the 600 m deeper transect. Actually, the higher local diversity at the shelf break compared to the upper slope seems to contrast with the regular observed bathymetric diversity gradients for the deep sea, with mid-slope diversity maxima recorded for multiple taxa (Levin and Dayton, 2009; Rex, 1981). However, the higher diversity at genus level for nematodes at the shelf break does not necessarily imply a higher species diversity for this environment, as some of the dominant deeper water genera such as *Acantholaimus* and *Halalaimus* are known as highly diverse genera (De Mesel et al., 2006; Muthumbi and Vincx, 1997). Still, generic diversity represents better the actual functional diversity for nematodes, as different genera are assumed to differ more in function than species within the same genus (Pape et al., 2013).

4.2. Greater sediment heterogeneity results in a higher beta diversity

In our study, distance-based linear models (DistLM) displayed a major significant effect of the silt-clay proportion on community variation when compared between the two depth transects (Fig. 7). The high silt-clay contribution, together with the low variation in sediment composition within and between stations of the 'deep' transect, was associated with a lower beta

diversity in this area (Fig. 5). As mentioned earlier, the stability of this environment seems to reduce the generic diversity, and favours the dominance of the genera *Acantholaimus* and *Halalaimus*, commonly abundant in deep-sea soft sediments (Vanreusel et al., 2010). Sediment particle-size diversity (SED) can be related to a higher settling speed of coarser sediments, which can quickly settle out (Condie and Sherwood, 2006). Usually, the augmentation of bottom stress is responsible for

5   eroding and suspending the local sediment, separating the dominant sandy fraction from the smaller silt-clay fraction, which remains in suspension (Quaresma et al., 2007). Consequently, if higher resuspension rates of sediment can occur, such as in the 'shallow' stations studied here, this will also affect resuspension of organisms dwelling at or within the sea bottom, while increasing the sediment variability. Near-bottom currents at the WIM, varying from 0.1–0.2 m s^{-1}, have the capacity to erode and suspend up to 0.01g m^{-2} s^{-1} of sediment, and are particularly strong at the shelf break (Condie and Sherwood, 2006; Drago

10   et al., 1998; Quaresma et al., 2007). In addition to bottom dynamics, the WIM is also known to be subjected to fisheries, which apply bottom trawls down to a depth of 800 m, affecting the continuous mixing and resuspension of surface sediments from the continental slope (ICES, 2008; Pusceddu et al., 2014).

Additionally to the homogeneous silty sediments, also the persistent low food availability in the 'deep' transect reduces the environmental variability, and may consequently limit the generic turnover. These results are concurrent with other

15   studies (Leduc et al., 2012b; Netto et al., 2005), where the same lack of correlation between beta diversity and organic matter has been observed also at species level (Leduc et al., 2012b). The relationship between food and regional diversity in the deep sea is still not well understood and seems to vary according to the different habitats studied. It appears that typical deep-sea communities dominated by similar genus composition can coexist in a food-depleted environment, while seemingly contrasting biodiversity at both local and regional scale increases with increasing food input. In this sense, when food input reaches a

20   certain threshold, more competitive/opportunistic species are going to rise and dominate the community, setting limit to the diversity of the system (Lins et al., 2015; Whittaker et al., 2001). This process may possibly explain why diversity in the deep sea is in general higher (at the slope) compared to coastal sediments (Moens et al., 2014), despite the higher food input in shallow waters. Nevertheless, in this study, the higher sediment heterogeneity at the shelf break accounted the most for the higher generic turnover between stations from both slope and shelf-break transects.

25

4.3. Beta diversity between different bathymetric transects is higher than beta diversity across similar depths

In general, dissimilarity in genus composition increased with both depth difference and geographical distance. However, dissimilarities between depth transects were larger than dissimilarities within transects, and genus turnover between the 'shallow' stations was greater than between the 'deep' stations (Fig. 5). If geographical distance would be an important

30   factor, one would also expect a similar high turnover for the deep transect as observed for the shallow transect. Concerning water depth, other studies on various taxa have shown that even small bathymetrical changes can be more important for promoting taxonomic differentiation than large geographical distances within the same depth (Havermans et al., 2013; Quattro et al., 2001). Nevertheless, the strong differences in sediment composition between transects (Figs. 5 and 7) explained 33 %

of the total nematode community variation between transects versus 3 % for water depth, appointing sediment differences as the main responsible factor for the higher beta diversity between transects rather than spatial differences. These findings suggest that diversity changes can be associated with both large and small-scale features driven by environmental alterations.

5   In general, beta diversity in the deep sea appears to be regulated by mechanisms of energy availability, biological interactions, disturbance, and habitat heterogeneity (Levin et al., 2001). Changes in these features at the slope occur much faster with increasing depth than with increasing isobathic distances (Rex, 1981), even for short distances, such as at the WIM study area. However here, still a considerate percentage of shared genera occurred between both transects and some genera exhibited similar abundances for both shelf break and upper slope, such as *Tricoma, Daptonema,* and *Halalaimus* (Fig. 3). In this respect, our results do not support the idea of isolation by depth between the shelf break and slope at the WIM area (Bik

10   et al., 2010; Riehl and Kaiser, 2012). To conclude, heterogeneity in sediment and possibly food availability to a lesser extent explain the main depth turnover patterns in this study, while depth and geographical distance are not the main cause for variations in community composition.

4.4. Absence of depth-specific clades

15   Although we observed distinct differences in community structure between the shallow and deep stations, the large proportion of genera shared between the two depth transects may indicate that bathymetrical isolation between the respective populations does not exist (Fig. 3). Yet, such depth-differentiation could occur on the intra-specific and species level. In our study, phylogenetic relationships within and between the genera sampled at both shallow and deep stations revealed shared clades (Fig. S1), potentially representing eurybathic species and thus at least some degree of connectivity across depths,

20   although we can make no conclusions about spatial or time scales. The precise understanding of spatial variability and the processes which drive species diversity and connectivity in the deep sea are presently still poorly understood (Danovaro et al., 2013; Etter and Bower, 2015). Deep basins are confluent at extensive depths and connected by thermohaline circulation, suggesting they do not represent completely isolated systems (Levin et al., 2001). Processes such as deep-water formation and upwelling, potentially represent means of (passive) across-depths dispersal (Brandt, 1992; Brandt et al., 2007; Kussakin, 1973;

25   Strugnell et al., 2008). Contrastingly, for some deep-sea taxa, such as protobranch bivalves, gastropods, and some crustaceans, depth-related diversification have been observed, indicating possible depth-related barriers to dispersal (Etter et al., 2011, 2005; Etter and Bower, 2015; Havermans et al., 2013; Wilson, 1983). The depth-related population differentiation observed in these studies, however, covered larger bathymetric ranges than the ones studied here, and were mostly situated at the lower bathyal and abyss (Etter et al., 2011; Etter and Bower, 2015). Just a few studies have assessed shallow-deep connectivity using

30   a combined morphological and molecular approach (Bik et al., 2010; Riehl and Kaiser, 2012; Van Campenhout et al., 2014; Van Gaever et al., 2009). In contrast to the molluscs and crustaceans mentioned above, selected nematodes and isopods show high degrees of genetic similarity across depth, suggesting taxon-specific barriers (Bik et al., 2010; Riehl and Kaiser, 2012).

14

Except for *Halalaimus*, all deep-sea nematode genera discussed in this study were sequenced for the first time. Although the relatively conserved 18S rDNA used here may not be the most suitable marker to assess dispersal, evolutionary rates of this gene are unknown for the nematode genera studied. Nevertheless, the presence of identical sequences between individuals from shallow and deep habitats (Fig. 8) provides hints towards dispersal between depths at relatively recent evolutionary time-scales. Our results for *Halalaimus* are in accordance with Bik et al. (2010), revealing multiple historic interchanges between habitats of different depth for multiple species. Likewise, no clear geographical structuring was observed in our phylogenetic tree, although this result could be biased due to limited taxon and geographic sampling. Whether nematode dispersal occurs passively through hydrodynamics or is active employing chemical cues and active swimming, exchange among marine nematode assemblages can be maintained both over large (> 500 km) and small (50–100 km) geographical distances. This explains the success of these benthic organisms as colonizers (Boeckner et al., 2009; Derycke et al., 2013; Gallucci et al., 2008) and strikingly confirms the unparalleled suitability of nematodes as a model organism regarding studies on connectivity and species turnover in the deep sea.

Even though our results indicate a link between shallow and deep habitats, other studies have suggested endemism in deep-sea habitats for nematodes (De Mesel et al., 2006; Van Campenhout et al., 2014). For example, *Halomonhystera disjuncta* was previously believed to occur in both shallow and deep habitats (Van Gaever et al., 2009), but a recent study based on 18S, COI, and ITS sequences showed that this species in fact constitutes two different lineages occupying deep and shallow environments, respectively (Van Campenhout et al., 2014). The bathymetric and geographic range was however broader than here.

Our phylogenetic results moreover highlight the scarcity of publically available DNA sequence data for deep-sea nematodes. For example, we present here the first sequence of the genus *Microlaimus* (no records in the GenBank, searched on 3 Dec 2015). Other genera are poorly represented in public sequence depositories (e.g. *Gammanema*: two 18S sequences, *Leptolaimus*: three 18S sequences, and *Richtersia*: two 28S sequences). The use of more specific and variable markers, such as the mitochondrial COI or the rDNA internal transcribed spacer, was not possible within this study due to low success rate of DNA amplification. Low success rates in PCR amplification are a known issue in deep-sea nematodes, but the causes are not well understood (Bik et al., 2010). Degradation of DNA may have occurred during sample processing and could be caused by increases in temperature.

Genetic structuring of shallow-water nematode populations was shown by Derycke et al. (2013) based on more variable markers (COI, ITS). They showed that despite being capable of long distance dispersal, nematodes may also show clear genetic differentiation at small-scales. In this study, we displayed the high dispersal capabilities and connectivity for nematodes, but those were not high enough to counteract community differentiation observed in the genera composition. Moreover, it is possible that only a small number of species show relatively high dispersal (gene flow), while other species may have limited dispersal abilities.

5. Conclusions

Sediment heterogeneity seemed to be the most important factor responsible for the greater variation in nematode community structure at all spatial levels studied: within stations, across stations from the same depth, and between depth transects. Deeper stations were uniformly dominated by silt-clay and exhibited low turnover and similar nematode communities typically found in deep-sea soft sediments, dominated by the genera *Acantholaimus* and *Halalaimus*. Fig. 9 summarizes the main patterns (a) and processes (b) by illustrating the increase in generic turnover from beta 1 ('deep transect) to beta 2 (shallow transect), and finally beta 3 (between both transects). High sediment variability at the shallow stations was associated to a more diverse and contrasting nematode community between stations, where high turnover was characterized by the dominance of different genera at each station. This high sediment heterogeneity at the shelf break suggests that strong near-bottom current pulses might be an indirect factor promoting diversity through the creation of patches and redistribution of resources, having thus positive effects on the benthic fauna. Moreover, the high beta diversity observed across transects surpassed the turnover at the shelf break and upper slope. These differences were attributed to the even higher differences in sediment variability observed between these two transects, with more diverse and variable sediments at the shallow transect, compared to the silt-clay dominance at the deep transect. This effect of sediment variability on the nematode community suggests that environmental factors were the main responsible for differences observed between stations and transects, minimizing the importance of geographical distance and depth as regulating factors. Nevertheless, despite the larger differences observed between transects, still a considerable percentage of shared genera was observed between the shelf break and the upper slope. Furthermore, phylogenetic clusters suggested that depth may not be a factor isolating populations of the nematode genus *Halalaimus*.

6. Acknowledgements

7. References

Akaike, H., 1981. A new look at the statistical-model identification. Curr. Contents/Engineering Technol. Appl. Sci. 22.

Alvarez-Salgado, X.A., Castro, C.G., Perez, F.F., Fraga, F., 1997. Nutrient mineralization patterns in shelf waters of the Western Iberian upwelling. Cont. Shelf Res. 17, 1247–1270. doi:10.1016/s0278-4343(97)00014-9

Amaro, T., Witte, H., Herndl, G.J., Cunha, M.R., Billett, D.S.M., 2009. Deep-sea bacterial communities in sediments and guts of deposit-feeding holothurians in Portuguese canyons (NE Atlantic). Deep. Res. I.

Anderson, R.N., Clarke, K.R., Gorley, M.J., 2007. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E.

5   Baldrighi, E., Lavaleye, M., Aliani, S., Conversi, A., Manini, E., 2014. Large Spatial Scale Variability in Bathyal Macrobenthos Abundance, Biomass, alpha- and beta-Diversity along the Mediterranean Continental Margin. PLoS One 9, 1–16. doi:http://dx.doi.org/10.1371/journal.pone.0107261

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L., 2008. GenBank. Nucleic Acids Research [WWW Document]. URL http://www.ncbi.nlm.nih.gov/genbank/

10   Bik, H.M., Thomas, W.K., Lunt, D.H., Lambshead, P.J.D., 2010. Low endemism, continued deep-shallow interchanges, and evidence for cosmopolitan distributions in free-living marine nematodes (order Enoplida). Bmc Evol. Biol. 10. doi:38910.1186/1471-2148-10-389

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C., 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature 302, 520–522. doi:10.1038/302520a0

15   Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T., Thomas, W.K., 1998. A molecular evolutionary framework for the phylum Nematoda. Nature 392, 71–75. doi:10.1038/32160

Boeckner, M.J., Sharma, J., Proctor, H.C., 2009. Revisiting the meiofauna paradox: dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. Hydrobiologia 624, 91–106.
20   doi:10.1007/s10750-008-9669-5

Brandt, A., 1992. Origin of Antarctic Isopoda (CRUSTACEA, MALACOSTRACA). Mar. Biol. 113, 415–423. doi:10.1007/bf00349167

Brandt, A., Gooday, A.J., Brandao, S.N., Brix, S., Broekeland, W., Cedhagen, T., Choudhury, M., Cornelius, N., Danis, B., De Mesel, I., Diaz, R.J., Gillan, D.C., Ebbe, B., Howe, J.A., Janussen, D., Kaiser, S., Linse, K., Malyutina, M.,
25   Pawlowski, J., Raupach, M., Vanreusel, A., 2007. First insights into the biodiversity and biogeography of the Southern Ocean deep sea. Nature 447, 307–311. doi:10.1038/nature05827

Cardinale, B.J., Nelson, K., Palmer, M.A., 2000. Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos 91, 175–183. doi:10.1034/j.1600-0706.2000.910117.x

Condie, S.A., Sherwood, C.R., 2006. Sediment distribution and transport across the continental shelf and slope under idealized
30   wind forcing. Prog. Oceanogr. 70, 255–270. doi:10.1016/j.pocean.2005.07.003

Crespo, B.G., Espinoza-Gonzalez, O., Teixeira, I.G., Castro, C.G., Figueiras, F.G., 2011. Possible mixotrophy of pigmented nanoflagellates: Microbial plankton biomass, primary production and phytoplankton growth in the NW Iberian upwelling in spring. Estuar. Coast. Shelf Sci. 94, 172–181. doi:10.1016/j.ecss.2011.06.008

Danovaro, R., Carugati, L., Corinaldesi, C., Gambi, C., Guilini, K., Pusceddu, A., Vanreusel, A., 2013. Multiple spatial scale
35   analyses provide new clues on patterns and drivers of deep-sea nematode diversity. Deep. Res. Part II-Topical Stud. Oceanogr. 92, 97–106. doi:10.1016/j.dsr2.2013.03.035

Danovaro, R., Company, J.B., Corinaldesi, C., D'Onghia, G., Galil, B., Gambi, C., Gooday, A.J., Lampadariou, N., Luna, G.M., Morigi, C., Olu, K., Polymenakou, P., Ramirez-Lledra, E., Sabbatini, A., Sarda, F., Sibuet, M., Tselepides, A., 2010. Deep-Sea Biodiversity in the Mediterranean Sea: The Known, the Unknown, and the Unknowable. PLoS One 5. doi:10.1371/journal.pone.0011832

5 De Grisse, A., 1969. Redescription ou modification de quelques techniques utilisés dans l'étude des nématodes phytoparasitaires. Meded. Rijksfakulteit Landbouwwet. Gent 34, 251–369.

De Mesel, I., Lee, H.J., Vanhove, S., Vincx, M., Vanreusel, A., 2006. Species diversity and distribution within the deep-sea nematode genus *Acantholaimus* on the continental shelf and slope in Antarctica. Polar Biol. 29, 860–871. doi:10.1007/s00300-006-0124-7

10 Derycke, S., Backeljau, T., Moens, T., 2013. Dispersal and gene flow in free-living marine nematodes. Front. Zool. 10. doi:110.1186/1742-9994-10-1

Derycke, S., Remerie, T., Vierstraete, A., Backeljau, T., Vanfleteren, J., Vincx, M., Moens, T., 2005. Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. Mar. Ecol. Prog. Ser. 300, 91–103. doi:10.3354/meps300091

15 Drago, T., Oliveira, A., Magalhaes, F., Cascalho, J., Jouanneau, J.M., Vitorino, J., 1998. Some evidences of northward fine sediment transport in the northern Portuguese continental shelf. Oceanol. Acta 21, 223–231. doi:10.1016/s0399-1784(98)80010-8

Easton, E.E., Thistle, D., 2016. Do some deep-sea, sediment-dwelling species of harpacticoid copepods have 1000-km-scale range sizes? Mol. Ecol. doi:10.1111/mec.13744

20 Etter, R.J., Bower, A.S., 2015. Dispersal and population connectivity in the deep North Atlantic estimated from physical transport processes. Deep. Res. Part I-Oceanographic Res. Pap. 104, 159–172. doi:10.1016/j.dsr.2015.06.009

Etter, R.J., Boyle, E.E., Glazier, A., Jennings, R.M., Dutra, E., Chase, M.R., 2011. Phylogeography of a pan-Atlantic abyssal protobranch bivalve: implications for evolution in the Deep Atlantic. Mol. Ecol. 20, 829–843. doi:10.1111/j.1365-294X.2010.04978.x

25 Etter, R.J., Grassle, J.F., 1992. Patterns of species-diversity in the deep sea as a function of sediment particle-size diversity. Nature 360, 576–578. doi:10.1038/360576a0

Etter, R.J., Rex, M.A., Chase, M.R., Quattro, J.M., 2005. Population differentiation decreases with depth in deep-sea bivalves. Evolution (N. Y). 59, 1479–1491. doi:10.1111/j.0014-3820.2005.tb01797.x

Figueiras, F.G., Labarta, U., Reiriz, M.J.F., 2002. Coastal upwelling, primary production and mussel growth in the Rias Baixas 30 of Galicia. Hydrobiologia 484, 121–131. doi:10.1023/a:1021309222459

Flach, E., Muthumbi, A., Heip, C., 2002. Meiofauna and macrofauna community structure in relation to sediment composition at the Iberian margin compared to the Goban Spur (NE Atlantic). Prog. Oceanogr. 52, 433–457. doi:Pii s0079-6611(02)00018-610.1016/s0079-6611(02)00018-6

France, S.C., Kocher, T.D., 1996. Geographic and bathymetric patterns of mitochondrial 16S rRNA sequence divergence 35 among deepsea amphipods, Eurythenes gryllus. Mar. Biol. 126, 633–643. doi:10.1007/bf00351330

Gage, J.D., 1997. High benthic species diversity in deep-sea sediments: The importance of hydrodynamics, Marine

Biodiversity: patterns and processes. Cambridge University Press, Cambridge.

Gallucci, F., Moens, T., Vanreusel, A., Fonseca, G., 2008. Active colonisation of disturbed sediments by deep-sea nematodes: evidence for the patch mosaic model. Mar. Ecol. Ser. 367, 173–183. doi:10.3354/meps07537

Garcia, R., Thomsen, L., 2008. Bioavailable organic matter in surface sediments of the Nazare canyon and adjacent slope (Western Iberian Margin). J. Mar. Syst. 74, 44–59. doi:10.1016/j.jmarsys.2007.11.004

Giere, O., 2009. Meiobenthology: the microscopic motile fauna of aquatic sediments.

Havermans, C., Sonet, G., D'Acoz, C. d'Udekem, Nagy, Z.T., Martin, P., Brix, S., Riehl, T., Agrawal, S., Held, C., 2013. Genetic and Morphological Divergences in the Cosmopolitan Deep-Sea Amphipod *Eurythenes gryllus* Reveal a Diverse Abyss and a Bipolar Species. PLoS One 8. doi:UNSP e74218 10.1371/journal.pone.0074218

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. 23, 399–489.

ICES, 2008. Report of the ICES Advisory Comittee (Report). ICES Advice.

Jennings, R.M., Etter, R.J., Ficarra, L., 2013. Population Differentiation and Species Formation in the Deep Sea: The Potential Role of Environmental Gradients and Depth. PLoS One 8. doi:UNSP e7759410.1371/journal.pone.0077594

Jensen, P., 1987. Feeding ecology of free-living aquatic nematodes. Mar. Ecol. Ser. 35, 187–196.

Jensen, P., 1981. Phyto-chemical sensitivity and swimming behavior of the free-living marine nematode *Chromadorita tenuis*. Mar. Ecol. Ser. 4, 203–206. doi:10.3354/meps004203

Katoh, K., Asimenos, G., Toh, H., 2009. Multiple Alignment of DNA Sequences with MAFFT (D Posada, Ed.). Bioinforma. DNA Seq. Anal. 537: 39–64.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. doi:10.1093/bioinformatics/bts199

Kussakin, O.G., 1973. Peculiarities of geographical and vertical distribution of marine Isopods and problem of deep-sea fauna origin. Mar. Biol. 23, 19–34. doi:10.1007/bf00394108

Lambshead, P.J.D., Tietjen, J., Ferrero, T., Jensen, P., 2000. Latitudinal diversity gradients in the deep sea with special reference to North Atlantic nematodes. Mar. Ecol. Prog. Ser. 194, 159–167. doi:10.3354/meps194159

Leduc, D., Rowden, A.A., Bowden, D.A., Nodder, S.D.S., Probert, P.K., Pilditch, C.C.A., Duineveld, G.G.C.A., Witbaard, R., 2012a. Nematode beta diversity on the continental slope of New Zealand : spatial patterns and environmental drivers. Mar. Ecol. Prog. Ser. 454, 37–52. doi:10.3354/meps09690

Leduc, D., Rowden, A.A., Probert, P.K., Pilditch, C.A., Nodder, S.D., Vanreusel, A., Duineveld, G.C.A., Witbaard, R., 2012b. Further evidence for the effect of particle-size diversity on deep-sea benthic biodiversity. Deep. Res. Part I-Oceanographic Res. Pap. 63, 164–169. doi:10.1016/j.dsr.2011.10.009

Lee, H.J., Vanhove, S., Peck, L.S., Vincx, M., 2001. Recolonisation of meiofauna after catastrophic iceberg scouring in shallow Antarctic sediments. Polar Biol. 24, 918–925. doi:10.1007/s003000100300

Levin, L.A., Dayton, P.K., 2009. Ecological theory and continental margins: where shallow meets deep. Trends Ecol. Evol. 24, 606–617. doi:10.1016/j.tree.2009.04.012

Levin, L.A., Etter, R.J., Rex, M.A., Gooday, A.J., Smith, C.R., Pineda, J., Stuart, C.T., Hessler, R.R., Pawson, D., 2001. Environmental influences on regional deep-sea species diversity. Annu. Rev. Ecol. Syst. 32, 51–93.

Lins, L., da Silva, M.C., Hauquier, F., Esteves, A.M., Vanreusel, A., 2015. Nematode community composition and feeding shaped by contrasting productivity regimes in the Southern Ocean. Prog. Oceanogr. 134, 356–369. doi:10.1016/j.pocean.2015.03.006

Lins, L., Guilini, K., Veit-Köhler, G., Hauquier, F., Alves, R.M.S., Esteves, A.M., vanreusel, A., 2014. The link between meiofauna and surface productivity in the Southern Ocean. Deep. Res. II 108, 60–68. doi:http://dx.doi.org/10.1016/j.dsr2.2014.05.003

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science (80-.). 294, 804–808. doi:10.1126/science.1064088

Lutz, M.J., Caldeira, K., Dunbar, R.B., Behrenfeld, M.J., 2007. Seasonal rhythms of net primary production and particulate organic carbon flux to depth describe the efficiency of biological pump in the global ocean. J. Geophys. Res. 112. doi:C1001110.1029/2006jc003706

McClain, C.R., Schlacher, T.A., 2015. On some hypotheses of diversity of animal life at great depths on the sea floor. Mar. Ecol. 1–24. doi:10.1111/maec.12288

Meldal, B.H.M., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., Austen, M.C., Blaxter, M.L., Rogers, A.D., Lambshead, P.J.D., 2007. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol. Phylogenet. Evol. 42, 622–636. doi:10.1016/j.ympev.2006.08.025

Miljutin, D.M., Miljutina, M.A., 2016. Review of *Acantholaimus* Allgén, 1933 (Nematoda: Chromadoridae), a genus of marine free-living nematodes, with a tabular key to species. Nematology 0, 1–22. doi:10.1163/15685411-00002976

Moens, T., Braeckman, U., Derycke, S., Fonseca, G., Gallucci, F., Gingold, R., Guilini, K., Ingels, J., Leduc, D., Vanaverbeke, J., Van Colen, C., Vanreusel, A., Vincx, M., 2014. Ecology of free-living marine nematodes, in: Schmidt-Rhaesa, A. (Ed.), Handbook of Zoology: Gastrotricha, Cycloneuralia and Gnathifera. Volume 2: Nematoda. pp. 109–152.

Moreno, M., Ferrero, T.J., Gallizia, I., Vezzulli, L., Albertelli, G., Fabiano, M., 2008. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuar. Coast. Shelf Sci. 77, 565–576. doi:10.1016/j.ecss.2007.10.016

Muthumbi, A.W., Vincx, M., 1997. *Acantholaimus* (Chromadoridae: Nematoda) from the Indian Ocean: description of seven species. Hydrobiologia 346, 59–76. doi:10.1023/a:1002937226161

Muthumbi, W.N.A., Vanreusel, A., Vincx, M., 2011. Taxon-related diversity patterns from the continental shelf to the slope: a case study on nematodes from the Western Indian Ocean. Mar. Ecol. 32, 453–467. doi:10.1111/j.1439-0485.2011.00449.x

Netto, S.A., Gallucci, F., Fonseca, G.F.C., 2005. Meiofauna communities of continental slope and deep-sea sites off SE Brazil. Deep. Res. Part I-Oceanographic Res. Pap. 52, 845–859. doi:10.1016/j.dsr.2004.11.009

Nolasco, R., Pires, A.C., Cordeiro, N., Le Cann, B., Dubert, J., 2013. A high-resolution modeling study of the Western Iberian Margin mean and seasonal upper ocean circulation. Ocean Dyn. 63, 1041–1062. doi:10.1007/s10236-013-0647-8

Pape, E., Bezerra, T.N., Jones, D.O.B., Vanreusel, A., 2013. Unravelling the environmental drivers of deep-sea nematode biodiversity and its relation with carbon mineralisation along a longitudinal primary productivity gradient. Biogeosciences 10, 3127–3143. doi:10.5194/bg-10-3127-2013

Posada, D., 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256. doi:10.1093/molbev/msn083

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818. doi:10.1093/bioinformatics/14.9.817

Puig, P., Canals, M., Company, J.B., Martin, J., Amblas, D., Lastras, G., Palanques, A., Calafat, A.M., 2012. Ploughing the deep sea floor. Nature 489, 286–+. doi:10.1038/nature11410

Pusceddu, A., Bianchelli, S., Martin, J., Puig, P., Palanques, A., Masque, P., Danovaro, R., 2014. Chronic and intensive bottom trawling impairs deep-sea biodiversity and ecosystem functioning. Proc. Natl. Acad. Sci. U. S. A. 111, 8861–8866. doi:10.1073/pnas.1405454111

Quaresma, L.S., Vitorino, J., Oliveira, A., da Silva, J.C.B., 2007. Evidence of sediment resuspension by nonlinear internal waves on the western Portuguese mid-shelf. Mar. Geol. 246, 123–143. doi:10.1016/j.margeo.2007.04.019

Quattrini, A.M., Baums, I.B., Shank, T.M., Morrison, C.L., Cordes, E.E., 2015. Testing the depth-differentiation hypothesis in a deepwater octocoral. Proc. R. Soc. B-Biological Sci. 282. doi:Unsp 2015000810.1098/rspb.2015.0008

Quattro, J.M., Chase, M.R., Rex, M.A., Greig, T.W., Etter, R.J., 2001. Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod Frigidoalvania brychia. Mar. Biol. 139, 1107–1113.

R Core Team, ., 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Raes, M., Rose, A., Vanreusel, A., 2010. Response of nematode communities after large-scale ice-shelf collapse events in the Antarctic Larsen area. Glob. Chang. Biol. 16, 1618–1631. doi:10.1111/j.1365-2486.2009.02137.x

Ramalho, S.P., Adao, H., Kiriakoulakis, K., Wolff, G.A., Vanreusel, A., Ingels, J., 2014. Temporal and spatial variation in the Nazare Canyon (Western Iberian margin): Inter-annual and canyon heterogeneity effects on meiofauna biomass and diversity. Deep. Res. Part I-Oceanographic Res. Pap. 83, 102–114. doi:10.1016/j.dsr.2013.09.010

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., Vecchione, M., 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences 7, 2851–2899. doi:10.5194/bg-7-2851-2010

Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., Peliz, A., da Silva, J.C.B., Santos, A.M.P., 2007. Physical oceanography of the western Iberia ecosystem: Latest views and challenges. Prog. Oceanogr. 74, 149–173. doi:10.1016/j.pocean.2007.04.021

Rex, M.A., 1981. Community structure in the deep-sea benthos. Annu. Rev. Ecol. Syst. 12, 331–353.

Rex, M.A., Etter, R.J., 2010. Deep-sea biodiversity: pattern and scale, Setting the stage: patterns of benthic standing stock. Harvard University Press.

Rex, M.A., McClain, C.R., Johnson, N.A., Etter, R.J., Allen, J.A., Bouchet, P., Waren, A., 2005. A source-sink hypothesis for abyssal biodiversity. Am. Nat. 165, 163–178.

Riehl, T., Kaiser, S., 2012. Conquered from the Deep Sea? A New Deep-Sea Isopod Species from the Antarctic Shelf Shows Pattern of Recent Colonization. PLoS One 7. doi:e4935410.1371/journal.pone.0049354

5   Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. doi:10.1093/bioinformatics/btg180

Rowe, G.T., Wei, C., Nunnally, C., Haedrich, R., Montagna, P., Baguley, J.G., Bernhard, J.M., Wicksten, M., Ammons, A., Escobar Briones, E., Soliman, Y., Deming, J.W., 2008. Comparative biomass structure and estimated carbon flow in food webs in the deep Gulf of Mexico. Deep. Res. Part II-Topical Stud. Oceanogr. 55, 2699–2711.
10   doi:10.1016/j.dsr2.2008.07.020

Salgueiro, E., Naughton, F., Voelker, A.H.L., de Abreu, L., Alberto, A., Rossignol, L., Duprat, J., Magalhaes, V.H., Vaqueiro, S., Turon, J.L., Abrantes, F., 2014. Past circulation along the western Iberian margin: a time slice vision from the Last Glacial to the Holocene. Quat. Sci. Rev. 106, 316–329. doi:10.1016/j.quascirev.2014.09.001

Salgueiro, E., Voelker, A.H.L., de Abreu, L., Abrantes, F., Meggers, H., Wefer, G., 2010. Temperature and productivity
15   changes off the western Iberian margin during the last 150 ky. Quat. Sci. Rev. 29, 680–695. doi:10.1016/j.quascirev.2009.11.013

Schoener, T.W., 1974. Resource partitioning in ecological communities. Science 185, 27–39. doi:10.1126/science.185.4145.27

Schratzberger, M., Whomersley, P., Warr, K., Bolam, S.G., Rees, H.L., 2004. Colonisation of various types of sediment by estuarine nematodes via lateral infaunal migration: a laboratory study. Mar. Biol. 145, 69–78. doi:10.1007/s00227-004-
20   1302-1

Serpetti, N., Gontikaki, E., Narayanaswamy, B.E., Witte, U., 2013. Macrofaunal community inside and outside of the Darwin Mounds Special Area of Conservation, NE Atlantic. Biogeosciences 10, 3705–3714. doi:10.5194/bg-10-3705-2013

Silvestro, D., Michalak, I., 2012. raxmlGUI: a graphical front-end for RAxML. Org. Divers. Evol. 12, 335–337. doi:10.1007/s13127-011-0056-0

25   Soetaert, K., Heip, C., 1995. Nematode assemblages of deep-sea and shelf break sites in the North Atlantic and Mediterranean Sea. Mar. Ecol. Ser. 125, 171–183. doi:10.3354/meps125171

Stachowicz, J.J., Bruno, J.F., Duffy, J.E., 2007. Understanding the effects of marine biodiversity on communities and ecosystems, in: Annual Review of Ecology Evolution and Systematics. pp. 739–766. doi:10.1146/annurev.ecolsys.38.091206.095659

30   Stamatakis, A., 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690. doi:10.1093/bioinformatics/btl446

Strugnell, J.M., Rogers, A.D., Prodohl, P.A., Collins, M.A., Allcock, A.L., 2008. The thermohaline expressway: the Southern Ocean as a centre of origin for deep-sea octopuses. Cladistics 24, 853–860. doi:10.1111/j.1096-0031.2008.00234.x

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version
35   6.0. Mol. Biol. Evol. 30, 2725–2729.

Thiel, H., 1978. Benthos in Upwelling Regions, Upwelling Ecosystems. Springer Berlin Heidelberg, Berlin. doi:10.1007/978-3-642-66985-9_11

Tokeshi, M., 1999. Species Coexistence: Ecological and evolutionary perspectives. Cambridge.

Ullberg, J., Olafsson, E., 2003. Free-living marine nematodes actively choose habitat when descending from the water column. Mar. Ecol. Ser. 260, 141–149.

Van Campenhout, J., Derycke, S., Tchesunov, A., Portnova, D., Vanreusel, A., 2014. The *Halomonhystera disjuncta* population is homogeneous across the Hakon Mosby mud volcano (Barents Sea) but is genetically differentiated from its shallow-water relatives. J. Zool. Syst. Evol. Res. 52, 203–216. doi:10.1111/jzs.12054

Van Gaever, S., Olu, K., Derycke, S., Vanreusel, A., 2009. Metazoan meiofaunal communities at cold seeps along the Norwegian margin: Influence of habitat heterogeneity and evidence for connection with shallow-water habitats. Deep. Res. Part I-Oceanographic Res. Pap. 56, 772–785. doi:10.1016/j.dsr.2008.12.015

Vanaverbeke, J., Bezerra, T.N., Braeckman, U., De Groote, A., de Meester, N., Deprez, T., Derycke, S., Gilarte, P., Guilini, K., Hauquier, F., Lins, L., Maria, T., Moens, T., Pape, E., Smol, N., Taheri, M., Van Campenhout, J., Vanreusel, A., Wu, X., vincx, M., 2015. Nemys: World Database of Free-living Marine Nematodes [WWW Document]. URL http://nemys.ugent.be

Vanreusel, A., Fonseca, G., Danovaro, R., da Silva, M.C., Esteves, A.M., Ferrero, T., Gad, G., Galtsova, V., Gambi, C., Genevois, V.D., Ingels, J., Ingole, B., Lampadariou, N., Merckx, B., Miljutin, D., Miljutina, M., Muthumbi, A., Netto, S., Portnova, D., Radziejewska, T., Raes, M., Tchesunov, A., Vanaverbeke, J., Van Gaever, S., Venekey, V., Bezerra, T.N., Flint, H., Copley, J., Pape, E., Zeppilli, D., Martinez, P.A., Galeron, J., 2010. The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. Mar. Ecol. Evol. Perspect. 31, 6–20. doi:10.1111/j.1439-0485.2009.00352.x

Vanreusel, A., Vincx, M., Van Gansbeke, D., Gijselinck, W., 1992. Structural Analysis of the meiobenthos communities of the shelf break area in two stations of the Gulf of biscay. Belgian J. Zool. 122, 185–202.

Vincx, M., Bett, B.J., Dinet, A., Ferrero, T., Gooday, A.J., Lambshead, P.J.D., Pfannkuche, O., Soltwedel, T., Vanreusel, A., 1994. Meiobenthos of the deep Northeast Atlantic. Adv. Mar. Biol. Vol 30 30, 1–88.

Warwick, H.M., Somerfield, P.J., Platt, R.M., 1998. Free-living marine nematodes part III - Monhysterids. Pictorial key to world genera and notes for the identification of British species.

Wei, C.-L., Rowe, G.T., Escobar-Briones, E., Boetius, A., Soltwedel, T., Caley, M.J., Soliman, Y., Huettmann, F., Qu, F., Yu, Z., Pitcher, C.R., Haedrich, R.L., Wicksten, M.K., Rex, M.A., Baguley, J.G., Sharma, J., Danovaro, R., MacDonald, I.R., Nunnally, C.C., Deming, J.W., Montagna, P., Levesque, M., Weslawski, J.M., Wlodarska-Kowalczuk, M., Ingole, B.S., Bett, B.J., Billett, D.S.M., Yool, A., Bluhm, B.A., Iken, K., Narayanaswamy, B.E., 2010. Global Patterns and Predictions of Seafloor Biomass Using Random Forests. PLoS One 5. doi:e1532310.1371/journal.pone.0015323

Whittaker, R.J., Willis, K.J., Field, R., 2001. Scale and species richness: towards a general, hierarchical theory of species diversity. J. Biogeogr. 28, 453–470. doi:10.1046/j.1365-2699.2001.00563.x

Wieser, W., 1953. Beziehungen zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Ark. für Zool. 4, 439–484.

Wilson, G.D., 1983. Variation in deep-sea Isopod *Eurycope iphthima* (ASELLOTA, EURYCOPIDAE) - Depth related clines

in rostral morphology and in population structure. J. Crustac. Biol. 3, 127–140. doi:10.2307/1547858

Wright, S.W., Jeffrey, S.W., 1997. High-resolution HPLC system for chlorophylls and carotenoids of marine phytoplankton, in: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (Eds.), Phytoplankton Pigments in Oceanography. UN Educational, Scientific and Cultural Organization, Paris, pp. 327–341.

5   Yoder, M., De Ley, I.T., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L., De Ley, P., 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology 8, 367–376. doi:10.1163/156854106778493448

Zeppilli, D., Vanreusel, A., Danovaro, R., 2011. Cosmopolitanism and Biogeography of the Genus Manganonema (Nematoda: Monhysterida) in the Deep sea. Animals 1, 291–305. doi:10.3390/ani1030291

10

Tables

Table 1. Sampling details

Station	Date	Depth (m)	Latitude	Longitude
St1	13/06/2013	445	37°58'959"N	09°11'050"W
St1	13/06/2013	445	37°58'953"N	09°11'094"W
St1	14/06/2013	445	37°58'967"N	09°11'090"W
St2	14/06/2013	335	37°58'904"N	09°07'525"W
St2	14/06/2013	335	37°58'913"N	09°07'528"W
St2	14/06/2013	335	37°58'888"N	09°07'513"W
St4	16/06/2013	325	37°51'171"N	09°06'944"W
St4	16/06/2013	325	37°51'188"N	09°06'974"W
St4	16/06/2013	325	37°51'174"N	09°06'950"W
St7	4/06/2014	295	37°47'448"N	09°05'430"W
St7	4/06/2014	294	37°47'494"N	09°05'442"W
St7	4/06/2014	290	37°47'490"N	09°05'380"W
St613	17/06/2013	296	37°55'597"N	09°06'998"W
St613	17/06/2013	298	37°55'594"N	09°07'001"W
St613	17/06/2013	298	37°55'594"N	09°07'002"W
St 614	3/06/2014	296	37°55'586"N	09°06'992"W
St 614	3/06/2014	294	37°55'455"N	09°06'934"W
St 614	3/06/2014	296	37°55'530"N	09°07'031"W
deep1	5/06/2014	906	37°58'871"N	09°23'061"W
deep1	5/06/2014	955	37°58'849"N	09°23'353"W

deep1	5/06/2014	955	37°58'752"N	09°23'405"W
deep1	6/06/2014	930	37°58'651"N	09°22'967"W
deep2	5/06/2014	939	37°54'163"N	09°25'150"W
deep2	5/06/2014	950	37°54'489"N	09°25'006"W
deep2	5/06/2014	900	37°54'550"N	09°24'994"W
deep2	5/06/2014	941	37°54'911"N	09°24'803"W
deep3	5/06/2014	996	37°51'450"N	09°27'752"W
deep3	5/06/2014	1001	37°51'650"N	09°27'615"W
deep3	5/06/2014	998	37°51'567"N	09°27'606"W
deep3	5/06/2014	950	37°51'595"N	09°27'324"W
deep4	5/06/2014	900	37°49'375"N	09°27'839"W
deep4	6/06/2014	957	37°49'661"N	09°28'042"W
deep4	6/06/2014	1006	37°49'307"N	09°28'214"W
deep4	6/06/2014	987	37°49'375"N	09°27'839"W

5

10

Figures
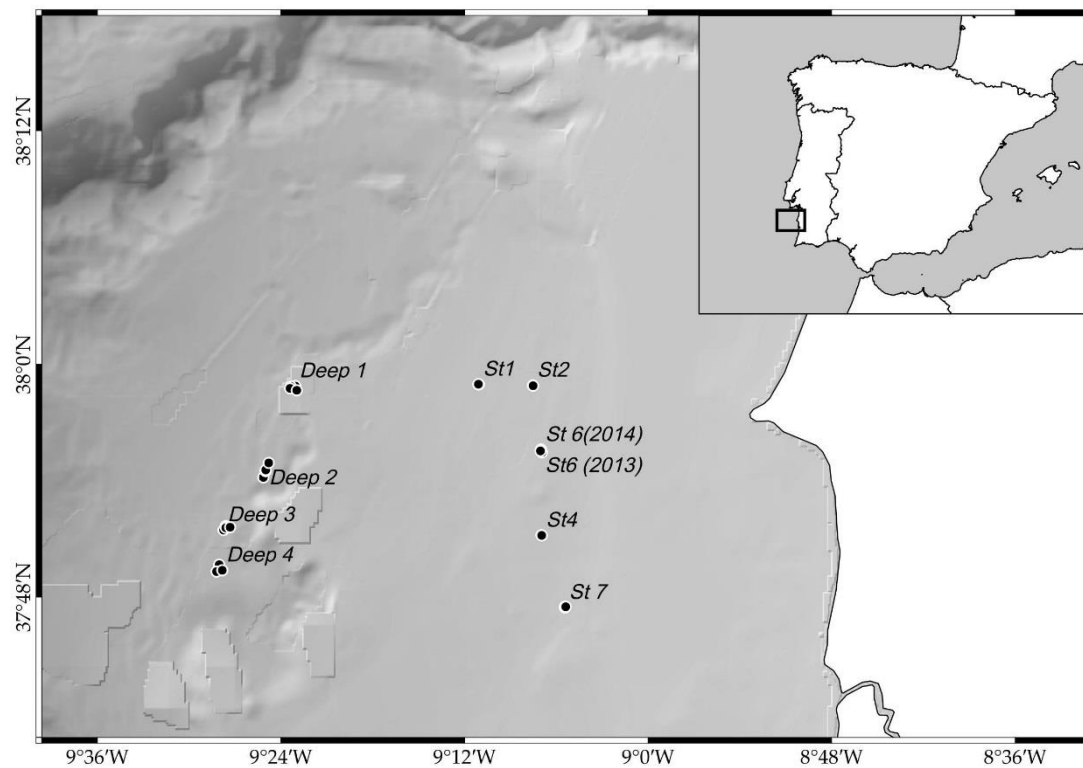
Figure 1. Location of B2013/17 and B2014/15 stations. Sampling stations are represented by black circles.

5
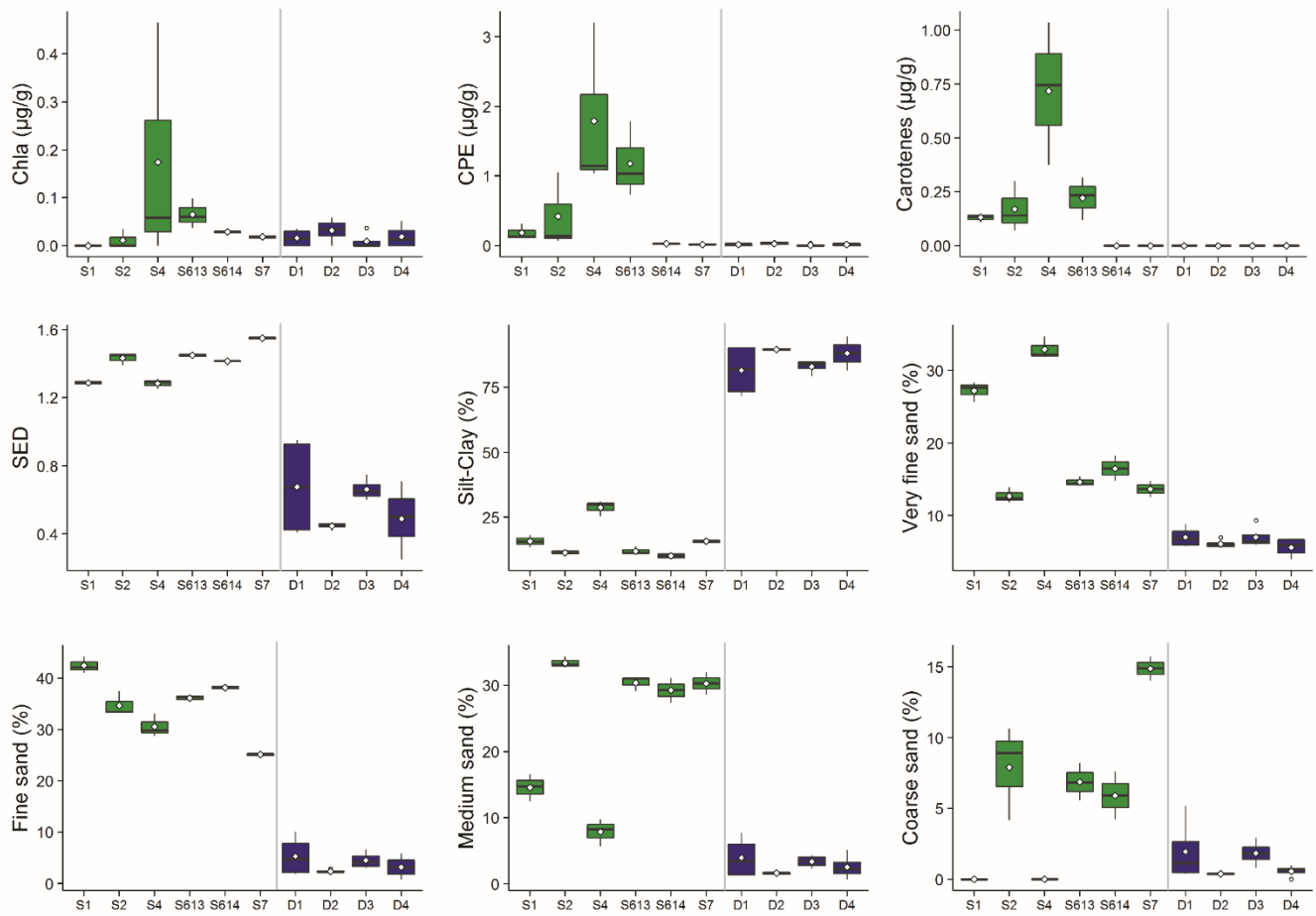
Figure 2. Environmental variables used in this study per station: Chl*a* (Chlorophyll a), CPE (Chloroplastic Pigment Equivalent), Carotenes, SED (sediment diversity), Silt-Clay, Very fine sand, Fine sand, Medium sand, and Coarse sand. Green boxplots represent shallow stations and blue boxplots represent deep stations. Black lines represent the median, empty circles represent the mean, lower box indicates the first quartile and upper box the third quartile. Upper line shows the maximum value and lower line the minimum value.
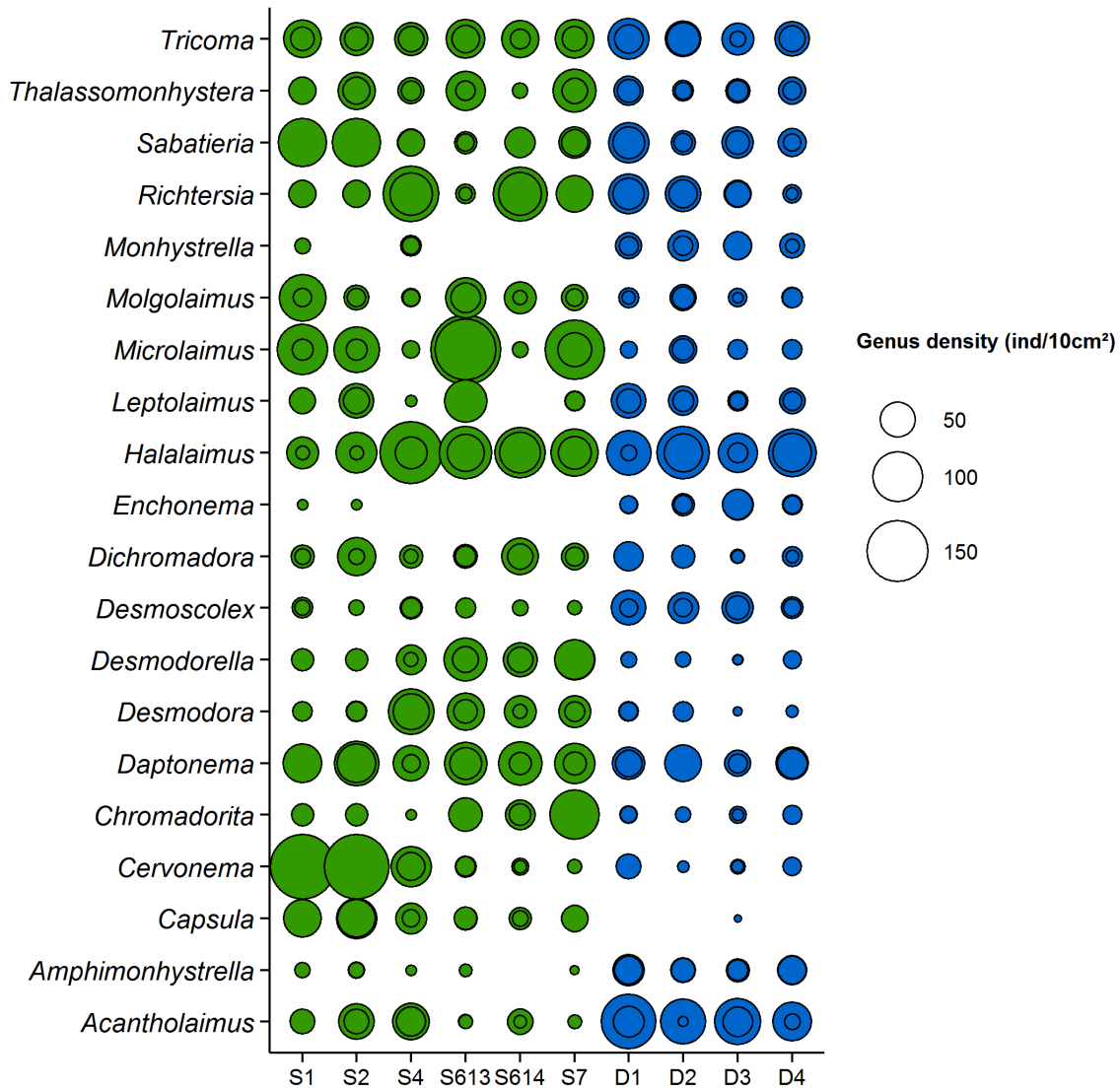
Figure 3. Most abundant nematode genera (> 4 %) per station. Inner circles indicate genus density (10 cm²) and outer circles genus standard deviation. Green circles represent shallow stations and blue circles deep stations.
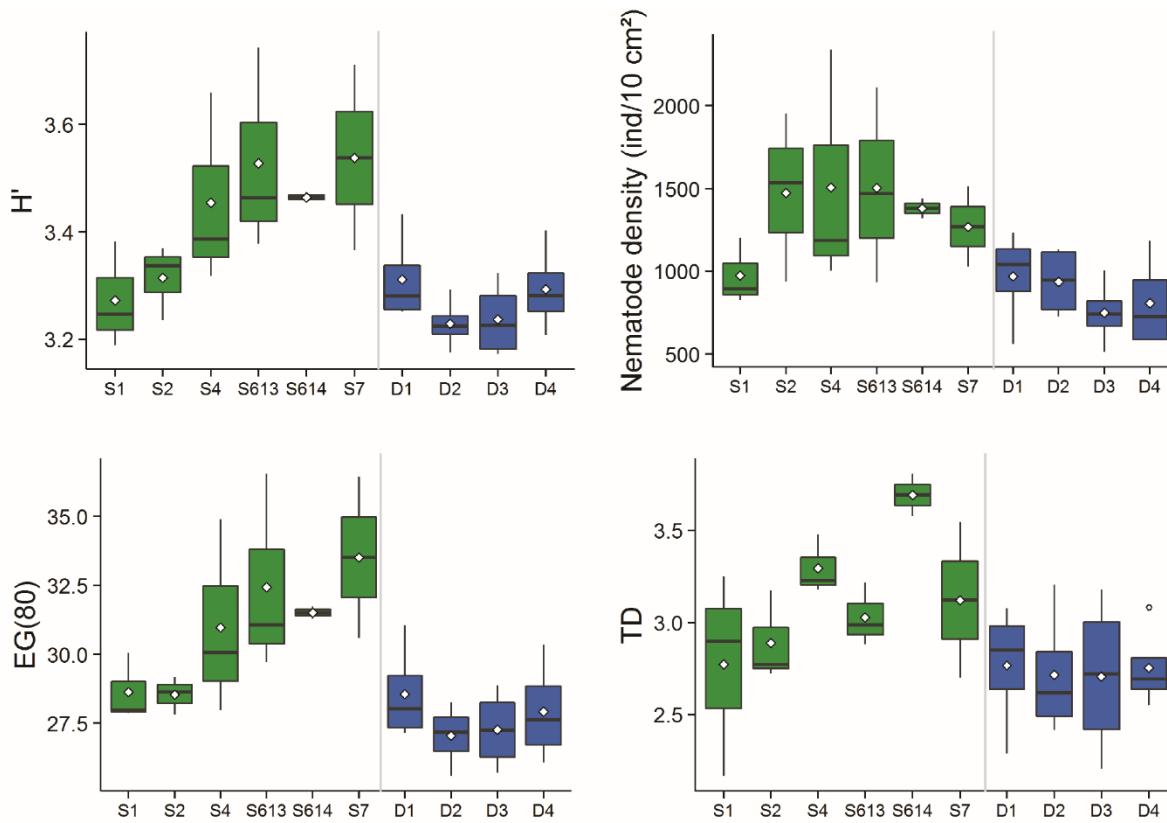
Figure 4. Nematode Shannon-Wiener (H') diversity, nematode density (individuals/10 cm²), Expected number of genera (EG(80)) and trophic diversity (TD) per station. Green boxplots represent shallow stations and blue boxplots represent deep stations. Black lines represent the median, empty circles represent the mean, lower box indicates the first quartile and upper box the third quartile. Upper line shows the maximum value and lower line the minimum value.
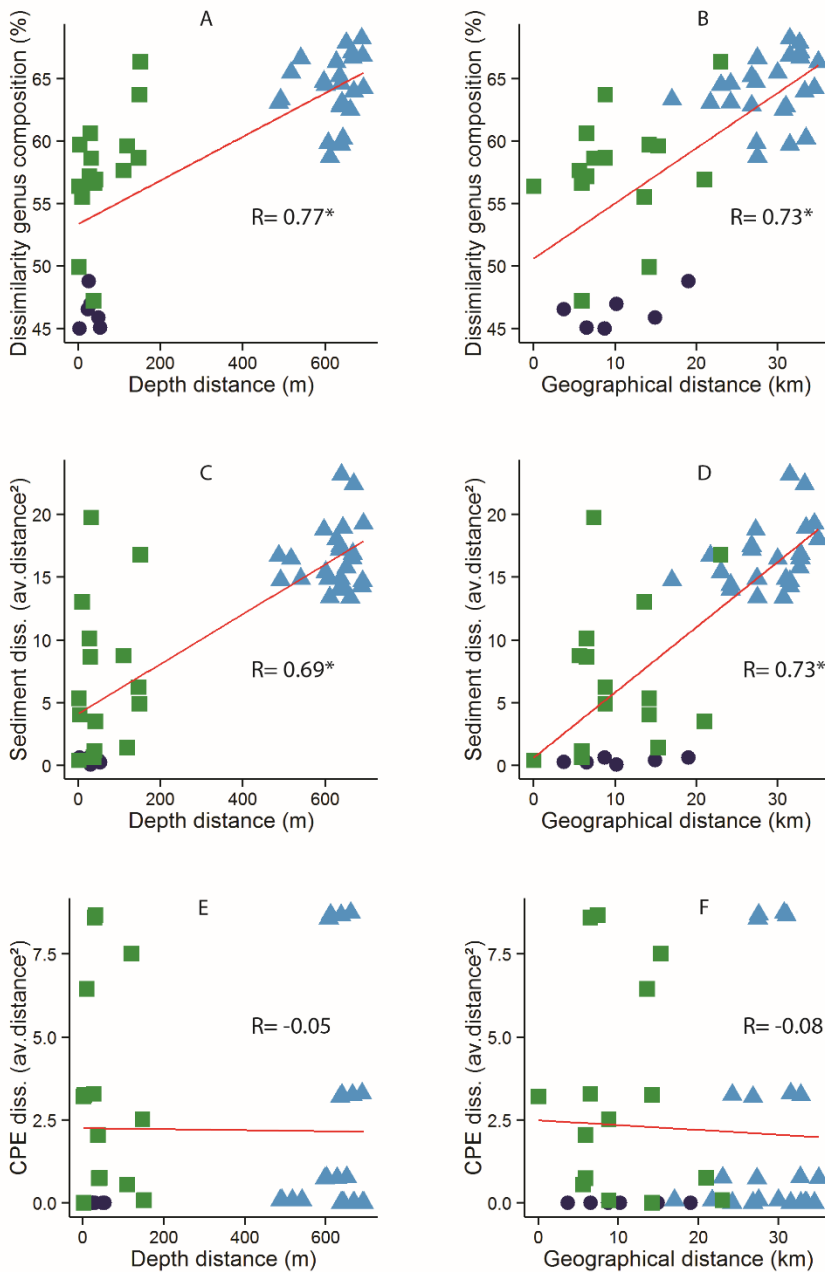
Figure 5. Dissimilarity values in nematode genus composition among depth difference (A) and between geographical areas (B), in sediment composition among depth difference (C) and between geographical areas (D), and in chloroplastic pigment equivalents (CPE) among depth difference (E) and geographical areas (F). Green squares represent dissimilarities between shallow stations, dark blue circles between deep stations and light blue triangles show dissimilarities between shallow and deep. Red lines indicate linear trends. . R indicate correlation values of Spearman. * shows significant correlations.
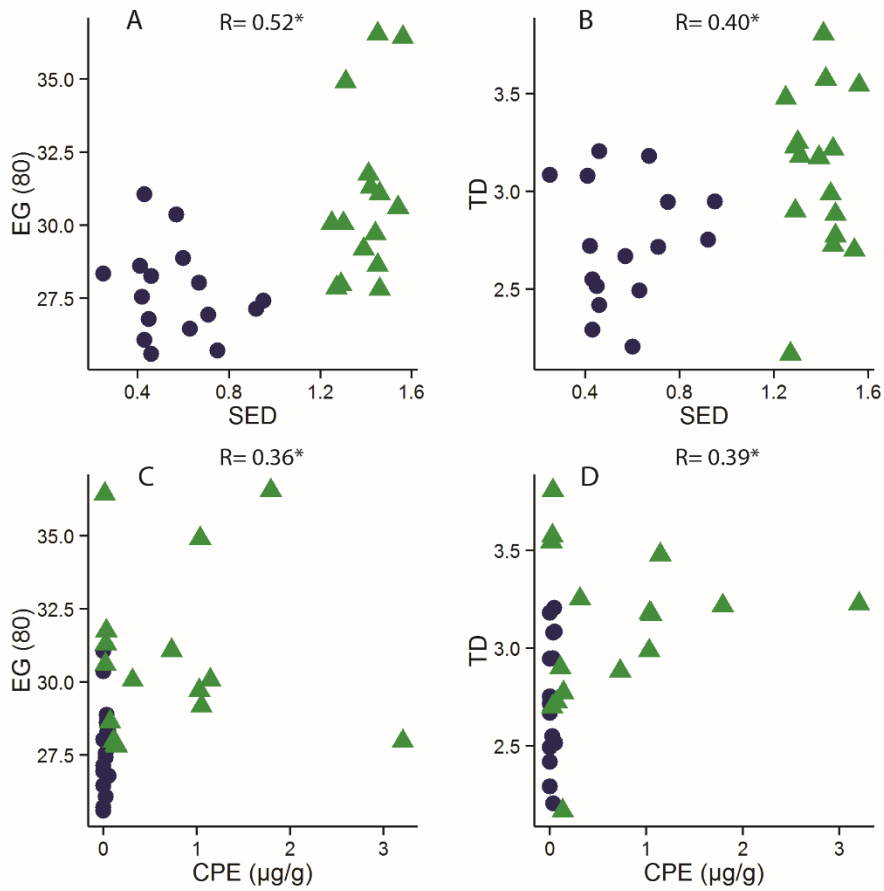
Figure 6. Correlations between (A) Sediment particle-size diversity (SED) and Expected genus diversity (EG (80)), (B) between SED and Trophic diversity (TD), (C) between EG (80) and Chloroplastic Pigment Equivalents (CPE) and (D) between TD and CPE. Green triangles represent correlations for shelf-break stations and dark blue circles between slope stations. R indicate correlation values of Spearman. * shows significant correlations.
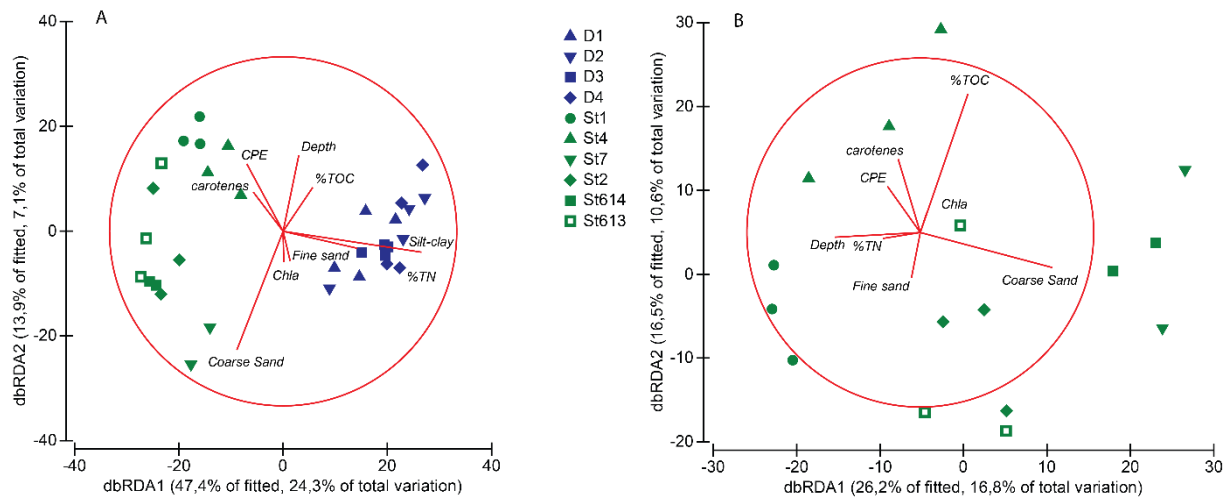
Figure 7. Distance-based redundancy analysis (dbRDA) illustrating the DistLM model based on the genera assemblage data for slope (blue) and shelf-break (green) stations (A) and only between shelf-break stations (B). Fitted environmental variables are shown with their vectors (strength and direction of effect of the variable on the ordination plot).
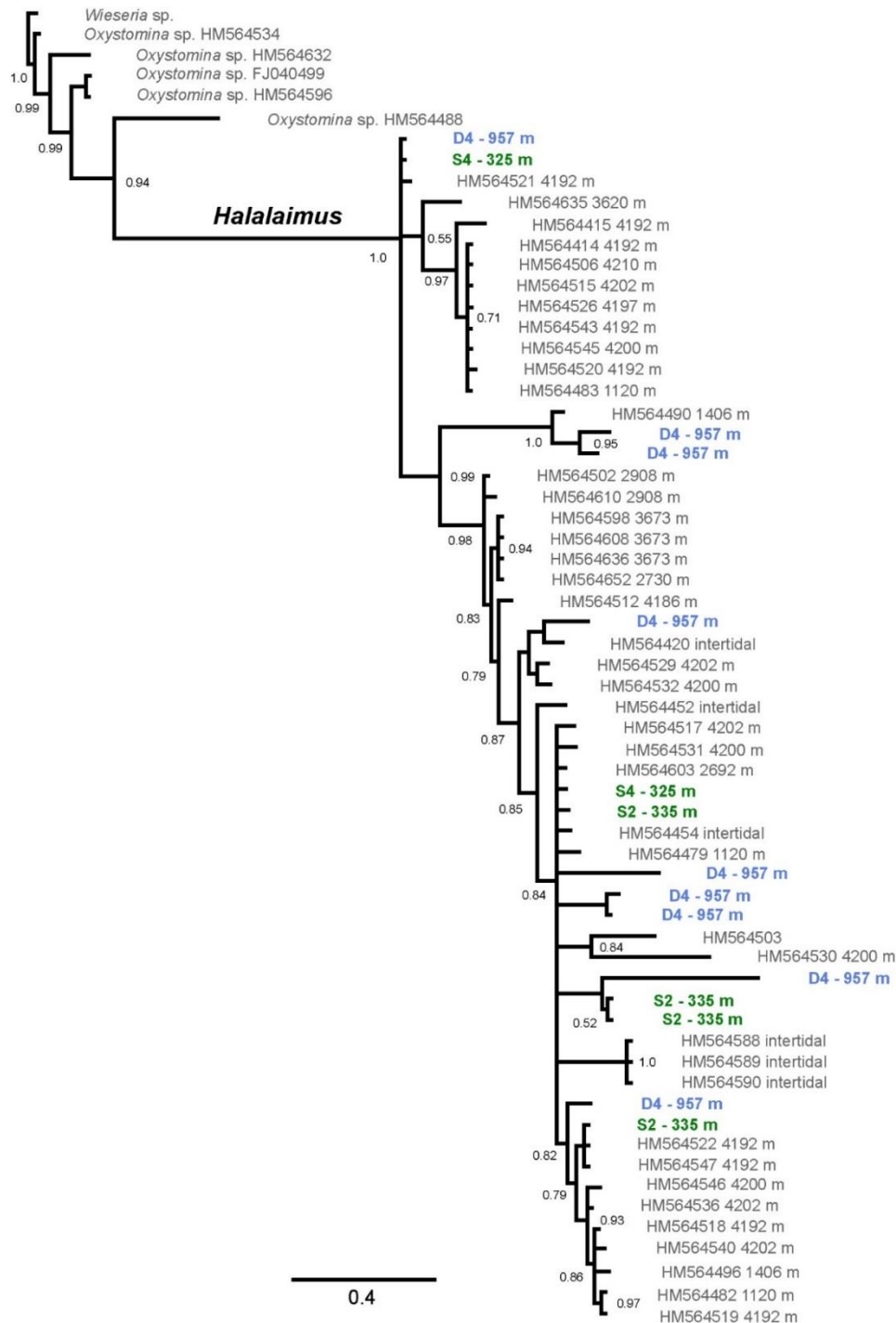
Figure 8. Consensus of Bayesian inference of phylogeny of the genus *Halalaimus* based on 18S rDNA sequence fragments generated in this study and from Bik et al. (2010); node support is given as posterior probabilities (PP); nodes with PP smaller than 0.50 were collapsed. The outgroup was set to *Wieseria*. The tree shows multiple instances of close relationships between individuals collected at different depth zones.
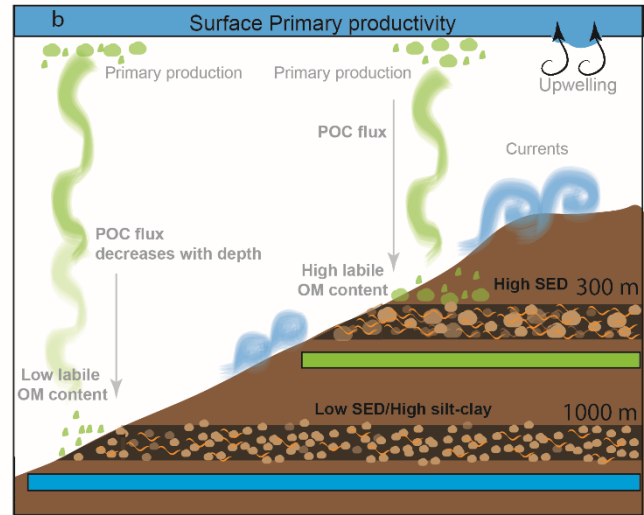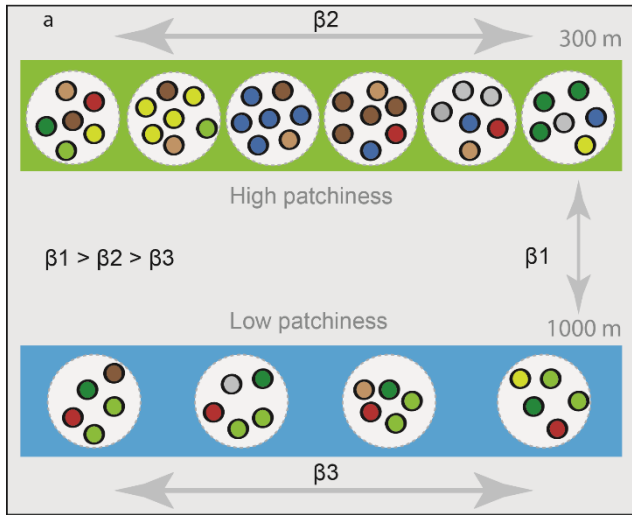
Figure 9. Scheme showing (a) how beta diversity varied across stations and between bathymetrical transects. The green bar represents the 'shallow' transect and the light blue bar the 'deep' transect. Grey circles inside the bars represents the stations sampled at each transect. Coloured circles inside grey circles refer to the variability in nematode genus composition within station and across stations. It illustrates the higher densities and patchiness found at the 'shallow' stations and the lower turnover found at the 'deep' stations. The (b) figure reveals the main environmental factors responsible for beta diversity between both depth transects. Upwelling effects, primary production, currents, and decrease of POC (particulate organic carbon) with increasing depth are considered to be correlated with both local and regional diversity. The fate of organic matter produced at the surface varies with depth, where deeper areas will receive lower labile organic matter (OM) when compared to shallower areas. This figure also illustrates the higher variability in sediment at the 'shallow' transect when compared to the deeper transect. The green bar represents the 'shallow' transect and the light blue bar the 'deep' transect.