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Dear Editor Marilaure Grégoire,

Enclosed is the manuscript entitled "Evaluating environmental drivers of spatial variability in free-living nematode assemblages along the Portuguese margin" by L Lins, F Leliaert, T Riehl, S P Ramalho, E Alfaro Cordova, A M Esteves, and A Vanreusel.

In the here presented manuscript we have addressed all the comments suggested by the last reviewer as well as the manuscript with track changes. We hope this revised version of the manuscript will be well received by the Editorial board of *Biogeosciences*.

Looking forward to hearing from you,

Yours sincerely,

Lidia Lins, on behalf of all authors

Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication)

Since being asked to review this manuscript, I have discussed my comments with the lead author personally –she is thus already aware of the comments I outline below. This is a nice study indeed with a design which I haven't seen applied to the deep sea before. However a number of modifications/additions would help the authors make the most of their data.

First, the authors need to very clearly define what they mean by diversity (eg what is meant by local diversity), and be very specific about the different spatial scales they investigate. This needs to be spelled out throughout the ms. For example online 32 it sounds as though the study is about comparing the importance of 4 scales, but only 2 (within and between transect) are (and can be) compared using statistics. Using the terms "within cores" is misleading and the authors should just say something like "alpha diversity at the scale of cores" –you get my drift.

The reviewer is right. The manuscript was revised and the level of diversity study clarified throughout the manuscript. Moreover, only differences between stations and between transects were tested statistically. Within-station diversity was studied through the SIMPER dissimilarities and their relationship visualized in scatter plots along a geographical gradient, as well as along sediment dissimilarity and CPE dissimilarity. The results and discussion sections were adapted to make these statements clearer.

"Diversity indices and nematode community structure turnover were investigated at three hierarchical levels: within stations, beta 1 (i.e. beta diversity between cores from the same station), between stations from the same depth, beta 2 (i.e. beta diversity between stations belonging to the same depth transect), and between two depth transects, beta 3. Turnover between stations from the same transect and between transects were statistically tested using the 2-factor nested PERMANOVA design described above. Dissimilarities within and between stations, and between transects were used to compare variability within each spatial scale."

The authors note that turnover diversity is much greater in the shallow transect. To test whether this is due to greater environmental heterogeneity at the shallow transect than deep transect, they need to do PERMDISP analysis on the multivariate environmental data between the two transects. If environmental variability is indeed greater in the shallow transect, then this could explain why the nematode communities are also more heterogeneous (i.e., have higher turnover among stations). It is easy to do and would yield further insights. This is a simpler and more elegant analysis (and thus more convincing) than the ones done at present.

We have conducted the analysis suggested by the reviewer and indeed we have observed that the variability in the environmental data was greater in the shallow transect when compared to the deep transect. We have incorporated these analyses in the manuscript. "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. PERMDISP analyses were conducted on the multivariate environmental data to investigate variability in the environmental conditions between the two transects. Subsequently, SIMPER routines were executed to identify which factors were responsible for between-transect differences."
"PERMDISP results revealed a higher variability (2.4 ±0.35 vs. 0.75 ± 0.087, after normalization of the data) in environmental conditions for the 'shallow' transect when compared with the 'deep' transect. According to the SIMPER results, these differences were mainly derived by higher average values of silt-clay and %TN in the 'deep' transect, and by the higher coarse sand content in the shallow transect, which together accounted for 45 % of the total dissimilarity."

I strongly disagree that Halalaimus is a deposit feeder. Their mouth cavity is practically non-existent. On what basis is this species classified as deposit feeder? Their buccal cavity is so different from say Daptonema and Sabatieria!

We have decided to delete this paragraph from the manuscript. Since no information is available neither for *Halalaimus* nor for *Acantholaimus,* I find that the discussion whether *Halalaimus* is a deposit feeder or microbial feeder is only speculation. I do not disagree with the reviewer that *Halalaimus* can be a microbial feeder, but evidence is still lacking concerning feeding ecology of this group. Moreover, it has already been shown for other animal groups that buccal morphology can be linked to feeding strategies, but that other animals can have similar buccal morphology and completely different feeding habits.

The geographical distance and depth "distance" (difference) graphs are ok but they both show very similar trends. This is because distance and depth difference are highly correlated (stations that are far apart tend to belong to different depth transects). This needs to be acknowledged in Discussion.

The reviewer is right. We have mentioned in the discussion that depth and geographical distances were correlated. However, the distances between transects and within each transect are quite similar (some shallow stations were even closer to some deep stations, see Fig. 1) and depth did not affect the community significantly according to the DistLM analysis. In addition, dissimilarity values have also increased within stations from the same transect (shallow) which were located at the same water depth. We have rebuilt the figure 5 according to the suggestion of the reviewer in order to show that the pattern found in geographical distance was very similar to the one found when dissimilarities in genus composition were plotted against sediment dissimilarity.
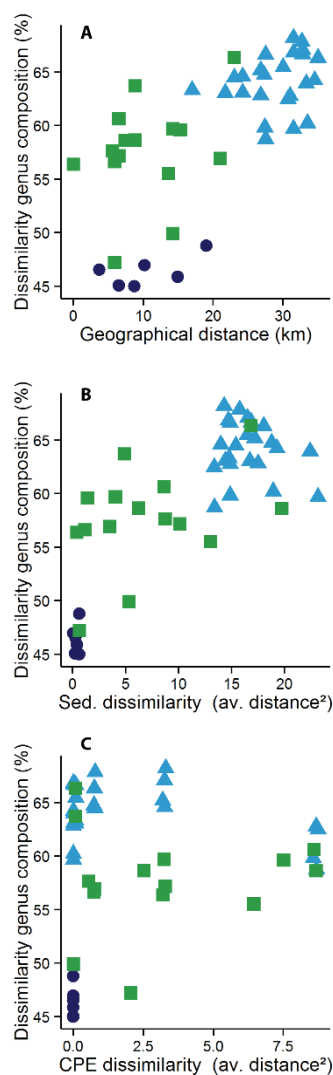
Figure 5. Dissimilarity values in nematode genus composition among geographical distance (A), sediment composition (B), and chloroplastic pigment equivalents (CPE). Green squares represent dissimilarities between shallow sites, dark blue circles between deep sites and light blue triangles show dissimilarities between shallow and deep.

Patterns observed within transects cannot be extrapolated to explain patterns between transects in the Discussion. For transects, N = 2, and differences in depth are correlated with a suite of other environmental factors and distance. So on needs to be careful when interpreting results.

In the DistLM analysis, depth effect on the nematode community was not significant neither for the marginal tests nor for the sequential tests. For this reason we believe differences observed between transects were due to environmental differences. However, the reviewer is right when he says we can only infer that depth did not have an effect on the nematode community. We have adapted the text in this section of the discussion accordingly.

"In general, dissimilarity in nematode genus structure increased with increasing geographical distance and DistLM results showed no significant effect of water depth on

the nematode community structure. However, genus turnover between the 'shallow' stations was greater than between the 'deep' stations. If geographical distance was an important factor, one would also expect a similar high turnover for the 'deep' transect as observed for the 'shallow' transect as both transects are equally long. 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, water depth did not have a significant effect on the differences observed for the nematode community structure in this study, and explained only 3 % of the total nematode community variation between transects, appointing environmental differences as the main responsible factors for the higher beta diversity between transects rather than spatial differences. These findings support the general idea that diversity changes can be associated with both large and small-scale features driven by environmental alterations.

"

I suggest incorporating some of Gray's results in relevant parts of ms (Gray et al. 1997 MEPS and Gray 2002 MEPS).

The results obtained by Gray were incorporated in the discussion according to the suggestion of the reviewer.

"Nevertheless, the relationship between food and beta 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 certain threshold, more competitive/opportunistic species are going to proliferate and dominate the community, setting a 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 (Gray, 2002; Moens et al., 2014), despite the higher food input in shallow waters. "

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

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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 rRegional generic diversity and turnover were investigated at four three 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.

Keywords: integrative taxonomy, habitat heterogeneity, dispersal, nematodes, Western Iberian Margin

1. Introduction

5   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

10   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

15   extraction (Levin and Dayton, 2009; Puig et al., 2012). The direct impact of these unabated commercial activities on the 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 pre-requisite for

20   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

25   habitat heterogeneity, and consequently promotes species coexistence (Cardinale et al., 2000). In addition, water 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

30   mostall 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). differences Beta diversity over large spatial scales (100–1000 m²; beta diversity) within continental margins have been observed both along

35   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

40   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, which belong~~ing~~ 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 geographical 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 history 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~~ for which repeated and regular interchanges between depths ~~were~~ have been 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 have combined bathymetric with geographic analyses along an isobathic transect 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~~ three spatial scales: ~~within a core,~~ within stations (i.e. beta diversity between cores from the same station), beta 1, between stations from the same depth (i.e. beta diversity between stations belonging to the same depth transect), beta 2, and between two depth transects, beta 3. 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 at small spatial scales (i.e. beta diversity between cores from the same station); (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~~ transect included six stations while the 'deep' ~~area~~ transect 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 size range) and divided into five categories, from silt-clay to coarse sand fractions. Sediment particle-size diversity (SED) was calculated as a measure for sediment heterogeneity 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 the~~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 (based on the 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):

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

where q_i is the relative abundance of trophic type i. Taxonomic diversity was measured using Shannon-Wiener diversity (H'), expected nematode genus richness (EG (80)) and Pielou's evenness (J'). Each replicate (core) value was used as a measurement of alpha diversity, while differences in genus structure within stations (i.e. between cores from the same site), between stations from the same transect, 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 untransformed 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 fixed 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 significant 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).

Diversity indices and nematode community structure turnover were investigated at three hierarchical levels: within stations, beta 1 (i.e. beta diversity between cores from the same station), between stations from the same depth, beta 2 (i.e. beta diversity between stations belonging to the same depth transect), and between two depth transects, beta 3. Turnover between stations from the same transect and between transects were statistically tested using the 2-factor nested PERMANOVA design described above. Dissimilarities within and between stations, and between transects were used to compare variability within each spatial scale.

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. PERMDISP analyses were conducted on the multivariate environmental data to investigate variability in the environmental conditions between the two transects. Subsequently, SIMPER routines were executed to identify which factors were responsible for between-transect differences. 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 R² 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 nematodes belonging to the 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~~ genus 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~~variables

5   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 compared to the 'shallow' 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~~ 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). Significantly higher values (nested PERMANOVA, $p < 0.05$) of % TOM (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 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 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 ($\geq$ 4 %) per station are ~~visualized~~ shown in Fig. 3. 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).

      The genera *Acantholaimus* and *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~~transects 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 '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 (Fig 4). Nested PERMANOVA results revealed

5 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 in nematode community structure between deployments within stations (beta 1) varied from 53–60

10 % (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 (beta 2) increased with increasing ~~depth and~~ geographical distance and sediment dissimilarity (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

15 depth ~~transects~~ (beta 3). The first genus was mainly found at 'deep' areas, whereas *Microlaimus* and *Richtersia* had higher densities at the '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], [S4, S7], ~~and~~ [S7, S2], and [S7, S613] possessed significant

20 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~~ transect (p > 0.05) (Fig. 4). Nested PERMANOVA results for relative abundance of trophic groups displayed significant differences between ~~depths~~the two transects, but not among stations from the same depth (p > 0.05) (Table S12). Average similarity between 'deep' and 'shallow' areas was 80 %.

25 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, %

30 TOC, Chla, carotenes, CPE, sediment grain size, and SED) are shown in Table S13. Evenness (J') was not correlated to any environmental factor. Shannon-Wiener ~~D~~diversity (H') was negatively correlated ~~to~~with 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~~with 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

35 sand, fine sand, and SED (Fig. 6).

PERMDISP results revealed a higher variability (2.4 ±0.35 vs. 0.75 ± 0.087, after normalization of the data) in environmental conditions for the 'shallow' transect when compared with the 'deep' transect. According to the SIMPER results, these differences were mainly derived by higher average values of silt-clay and %TN in the 'deep' transect, and by the higher coarse sand content in the shallow transect, which together accounted for 45 % of the total dissimilarity. DistLM

40 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~~ Beta diversity within and between stations (beta 1 and 2) from the same transect along the Western Iberian Margin (WIM) were higher at the shelf break ('shallow' stations) when compared with~~to~~ the upper slope ('deep' stations). The lower within and between-station turnover observed at the upper slope occurred because nematode communities were in general dominated by the same genera (*Halalaimus* and *Acantholaimus*), while at the shelf break not only a higher beta diversity was present ~~per~~ within stations, but also the genus composition clearly differed between stations, resulting in a higher turnover. This higher within and between-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 ~~from the 'shallow' transect~~. However, the largest community differences and therefore highest taxonomic turnover in nematode genera was present between ~~both depth~~ transects (beta 3), supported by the higher dissimilarity values observed ~~between transects~~ compared with within-transect variability. Nevertheless, ~~while~~ the high number of shared genera between transects and the intermingled pattern of genetic clustering observed for *Halalaimus* suggested that depth ~~as such~~differences 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~~ beta diversity

In this study, ~~local~~ nematode beta diversity within and between stations was significantly correlated with food availability. Moreover, the higher amount and variability (i.e. dissimilarities within and between stations from the same transect) 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 structural and trophic 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 variability of environmental variables, such as patchiness of food input and habitat heterogeneity, observed here, and consequently the high nematode beta~~local~~ diversity ~~(alpha and beta~~ within and between stations~~)~~ 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 general lower amount and within and between-station variability of food resources were associated with a ~~decline in~~lower nematode beta 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.

Nevertheless, the relationship between food and beta 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 certain threshold, more competitive/opportunistic species are going to proliferate and dominate the community, setting a 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 (Gray, 2002; Moens et al., 2014), despite the higher food input in shallow waters.

~~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 within and between-station beta~~local~~ diversity at the slope stations compared to the shelf break is not only explained by the lower and less patchy food input. In this study, beta diversity within and between stations

increased with increasing sediment dissimilarity and ~~S~~sediment heterogeneity (SED). Furthermore~~, , with~~ SED ~~was also revealing is~~showed positively correlate~~d values~~d 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 and CPE (Fig 6). In addition, distance-based linear models (DistLM) and SIMPER results based on the environmental data revealed a major significant effect of the silt-clay proportion on community beta diversity 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 within and between stations in this area (Fig. 5). In addition, ~~T~~the lower trophic diversity (TD) and higher ~~relative abundances~~contribution of deposit feeders in the deep stations ~~already mentioned above~~, were potentially caused by a combination ~~between~~ of low food input and comparatively higher sediment stability for this transect, reflected by the finer sediment composition in ~~comparison~~relation ~~with~~to the 'shallow' stations (Fig. 2). ~~As mentioned earlier, t~~The stability of the 'deep' transect~~is 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). 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).

In this regard, the homogeneous silty sediments and the persistent low food availability along the 'deep' transect might reduce environmental variability, and may consequently limit the genus turnover within and between stations there. Contrastingly,

~~The~~ the higher within and between-station trophic diversity at the 'shallow' stations suggests a higher niche differentiation within this transect and~~, 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~~within and between-station beta diversity 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 higher sediment heterogeneity and food patchiness at the stations located at the shelf break, are used to explain the higher within and between-station diversity compared to the 600 m deeper transect. Actually, the higher ~~local~~within and between-station beta 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~~ shelf-break stations 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~~may better represent 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 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 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 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 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 (Gray, 2002; 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.

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

In general, dissimilarity in nematode genus composition structure increased with both depth difference and increasing geographical distance and. DistLM results showed no significant effect of water depth on the nematode community structure. 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 was an important factor, one would also expect a similar high turnover for the 'deep' transect as observed for the 'shallow' transect as both transects they are equally long. 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, water depth did not have a significant effect on the differences observed for the nematode community structure in this study, and the strong differences in sediment composition between transects (Figs. 5 and 7) explained only 33 % of the total nematode community variation between transects, versus 3 % for water depth, appointing sediment environmental differences as the main responsible factors for the higher beta diversity between transects rather than spatial differences. These findings support suggest the general idea that diversity changes can be associated with both large and small-scale features driven by environmental alterations.

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, like the WIM study a~~ied area~~, ~~occur much faster~~are more pronounced 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 considerab~~le~~te percentage of shared

5   genera occurred ~~between~~at 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 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.~~3.   Absence of depth-specific clades

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

15   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, 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,

20   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; 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

25   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 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.,

30   2010; Riehl and Kaiser, 2012).

Except for *Halalaimus*, all deep-sea nematode genera ~~discussed~~ sequences obtained 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

35   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)

40   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 spatial scales. In this study, we ~~displayed~~ demonstrated the high dispersal capabilities and connectivity for nematodes, but those were not high enough to counteract community differentiation observed in the gen~~us~~era composition. Moreover, it is ~~possible~~ likely 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~~within and between stations from the same ~~depth~~transect, as well as~~nd~~ between ~~depth~~ transects. 'Deep'~~er~~ stations were uniformly dominated by silt-clay and exhibited low nematode turnover and ~~similar~~ less variable 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 (beta diversity within and between stations from the 'deep' transect) to beta 2 (beta diversity within and between stations from the 'shallow' transect), and finally beta 3 (beta diversity between both transects). High sediment variability at the 'shallow' stations was associated ~~to~~with a more diverse and ~~contrasting~~ variable 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 at the scale of transect through the creation of patches and redistribution of resources~~, having thus positive effects on the benthic fauna~~. Moreover, the high beta diversity observed ~~across~~ between transects surpassed the turnover ~~at~~within the shelf break and upper slope transects. 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~~rather than geographical distance ~~or~~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~~Finally, phylogenetic clusters suggested that depth may not be a factor isolating populations of the nematode genus *Halalaimus*.

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Tables

Table 1. Sampling details. St codes refer to sites sampled at the 'Shallow transect', while Deep codes refer to sites sampled at the 'Deep transect'. Each St site is characterized by three independent replicates obtained from three different deployments (three cores), while the Deep stations are characterized by four independent replicates obtained from four different deployments (four cores).

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

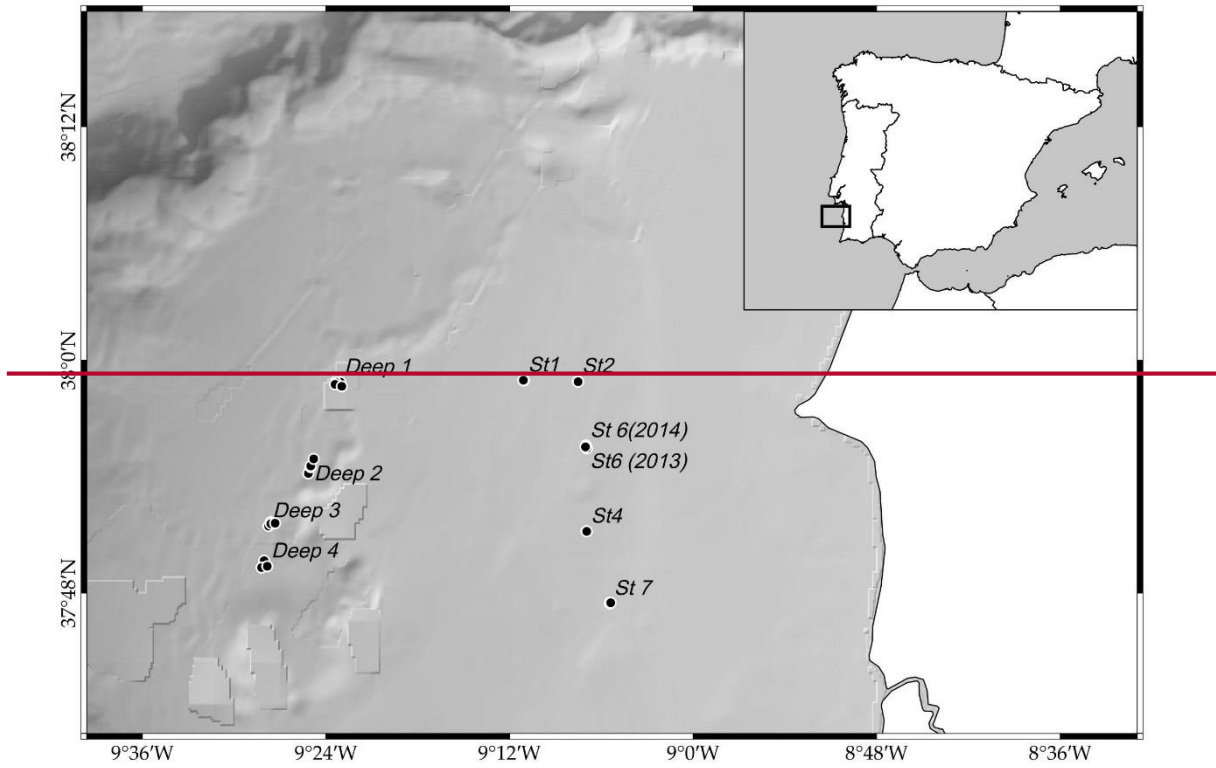
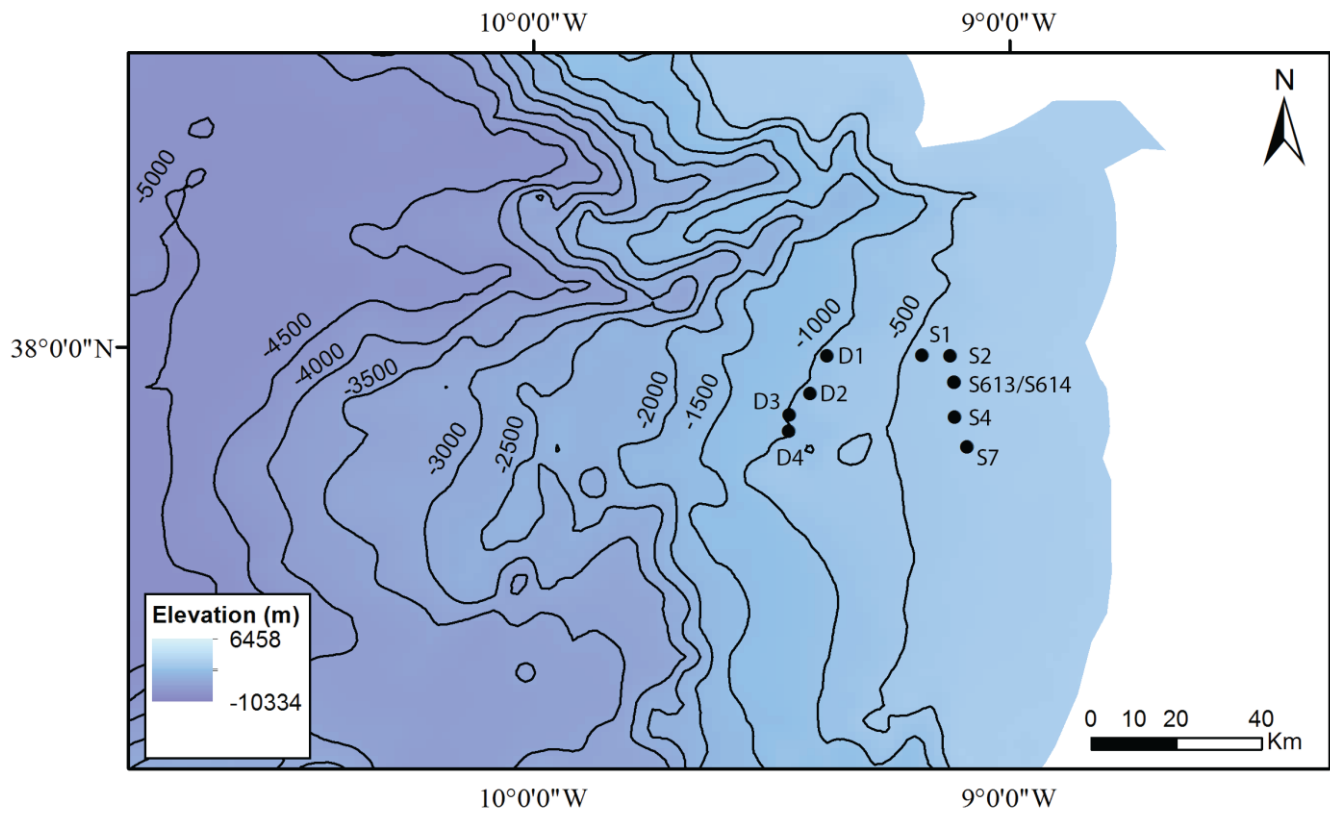
deep4	6/06/2014	957	37°49'661"N	09°28'042"W
deep4	6/06/2014	1006	37°49'307"N	09°28'214"W
deep4	6/06/2014	987	37°49'375"N	09°27'839"W

Figures

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

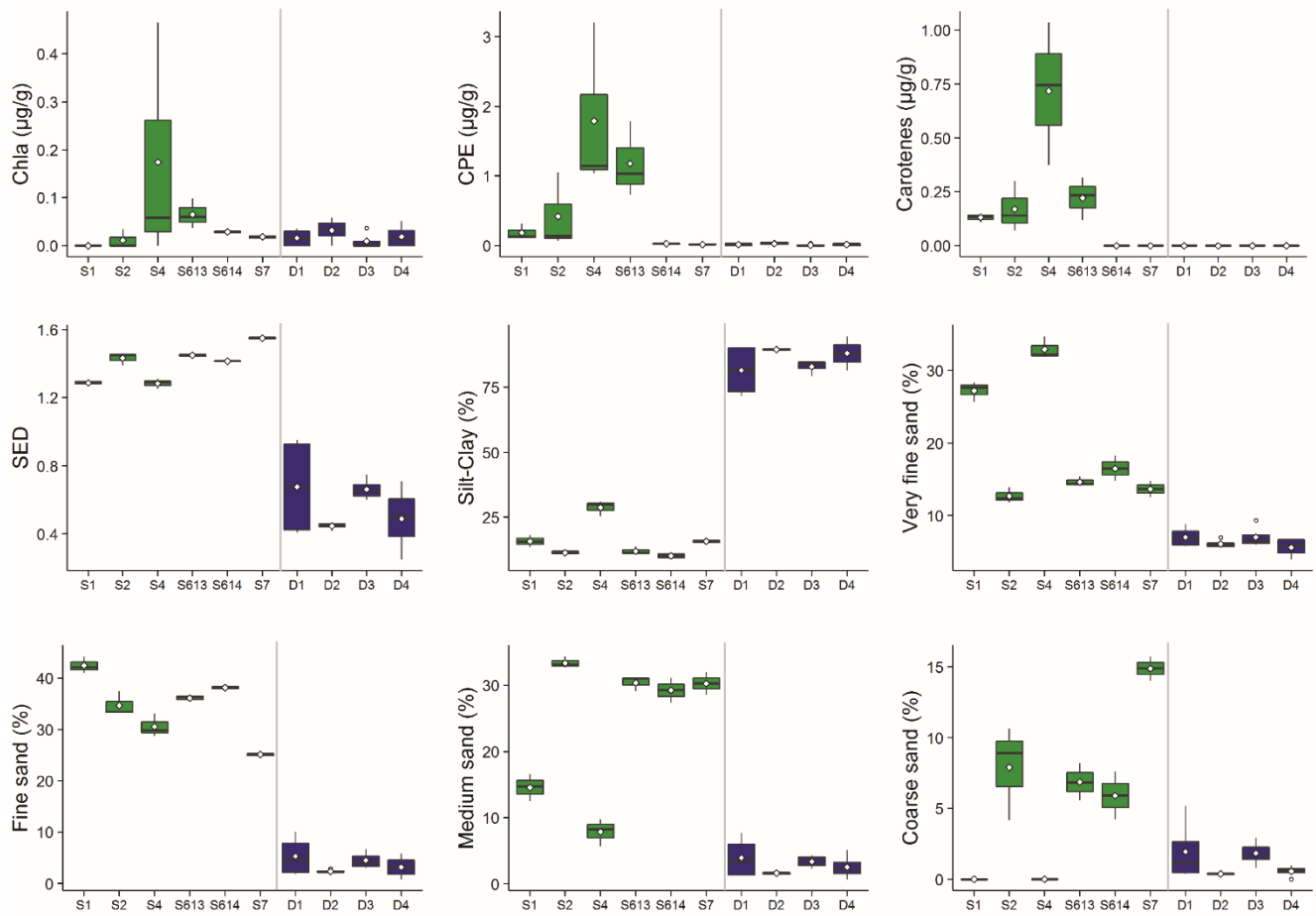



Figure 2. Environmental variables used in this study per station: Chl*a* (Chlorophyll a), CPE (Chloroplastic Pigment Equivalent), Carotenes, SED (sediment diversity), Silt-Clay, Very fine sand, Fine sand, Medium sand, and Coarse sand. Green boxplots represent shallow stations and blue boxplots represent 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.

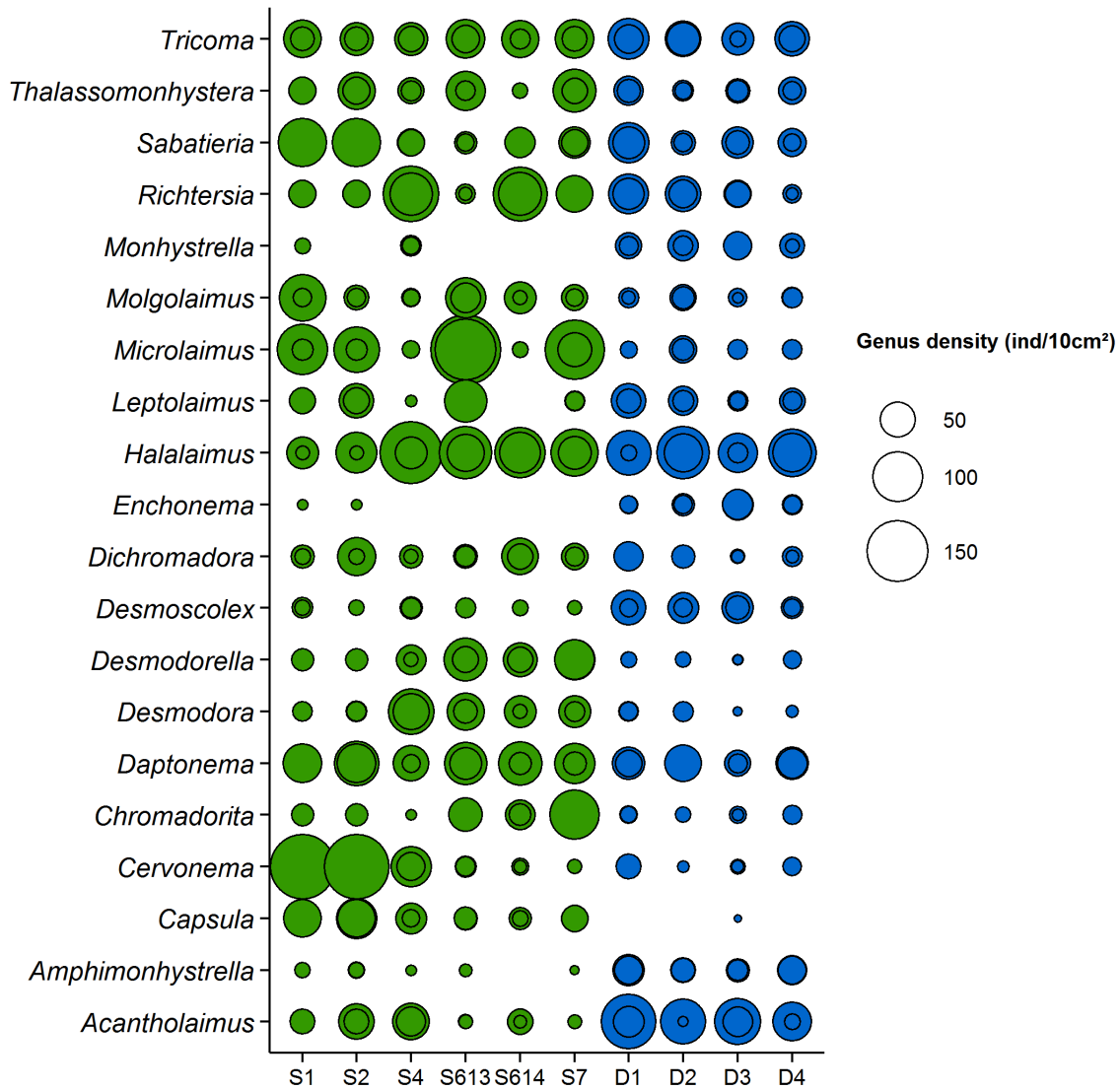



Figure 3. Most abundant nematode genera (> 4 %) per station. Inner circles indicate genus density (10 cm²) and outer circles genus standard deviation. Green circles represent shallow stations and blue circles deep stations.

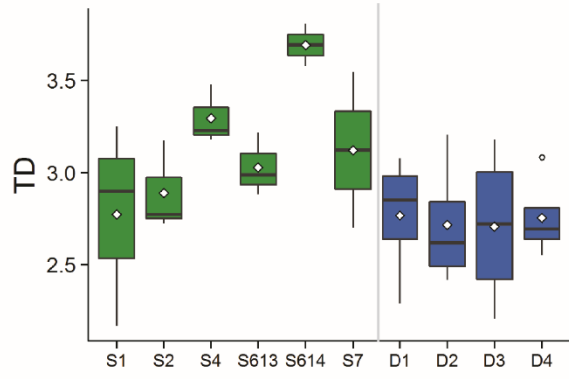
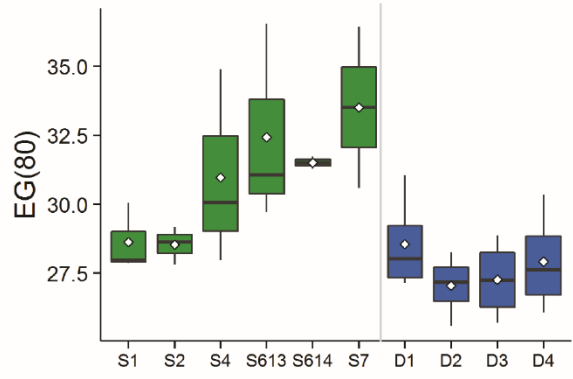
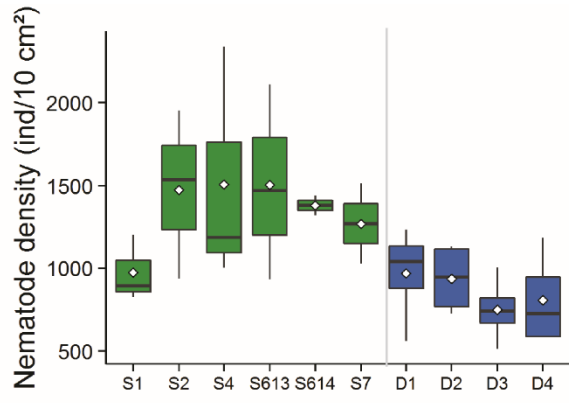
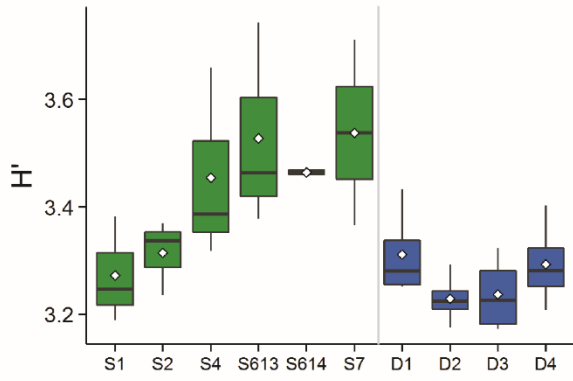
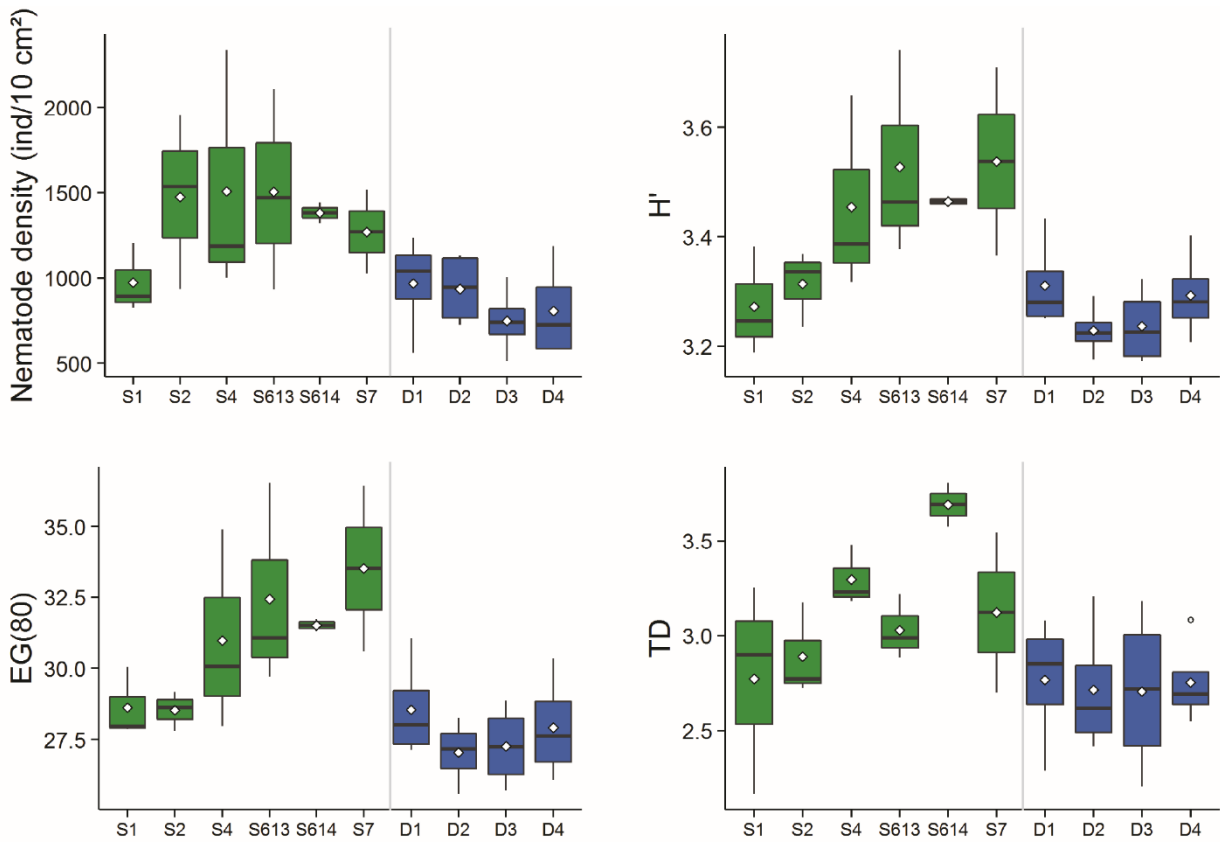



Figure 4. Nematode Shannon-Wiener (H') diversity, nematode density (individuals/10 cm²), Expected number of genera (EG(80)) and trophic diversity (TD) per station. Green boxplots represent shallow stations and blue boxplots represent deep stations. Black lines represent the median, empty circles represent 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.

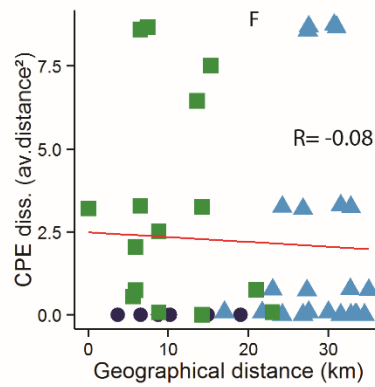
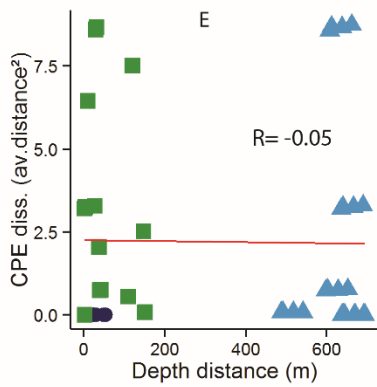
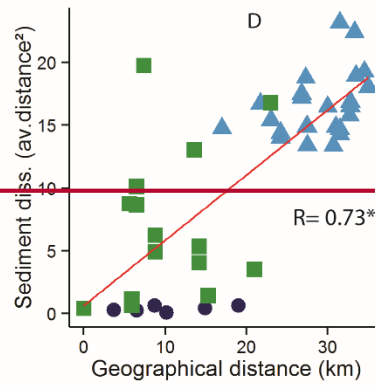
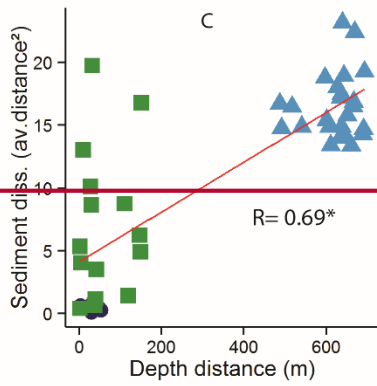
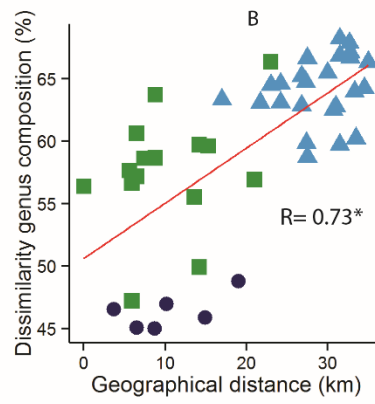
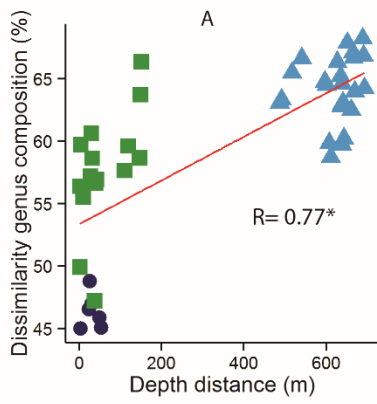
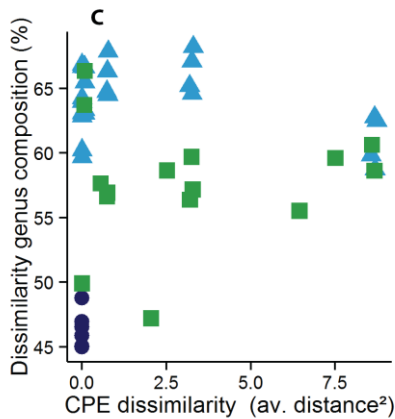
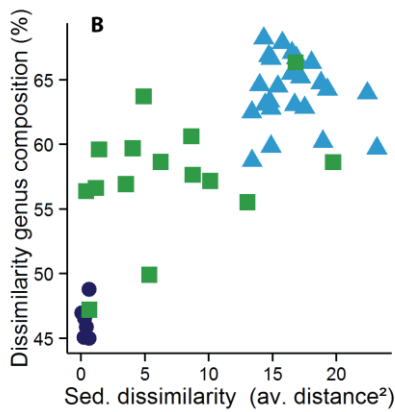
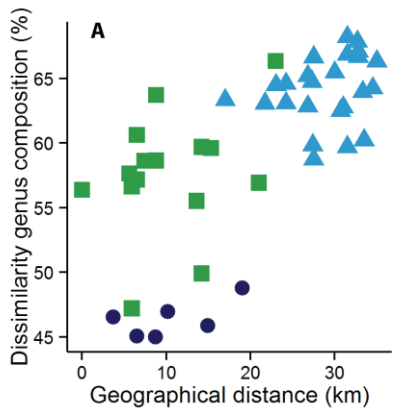



Figure 5. Dissimilarity values in nematode genus composition among geographical distance (A), sediment composition (B), and chloroplastic pigment equivalents (CPE). Green squares represent dissimilarities between shallow sites, dark blue circles between deep sites and light blue triangles show dissimilarities between shallow and deep.

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.

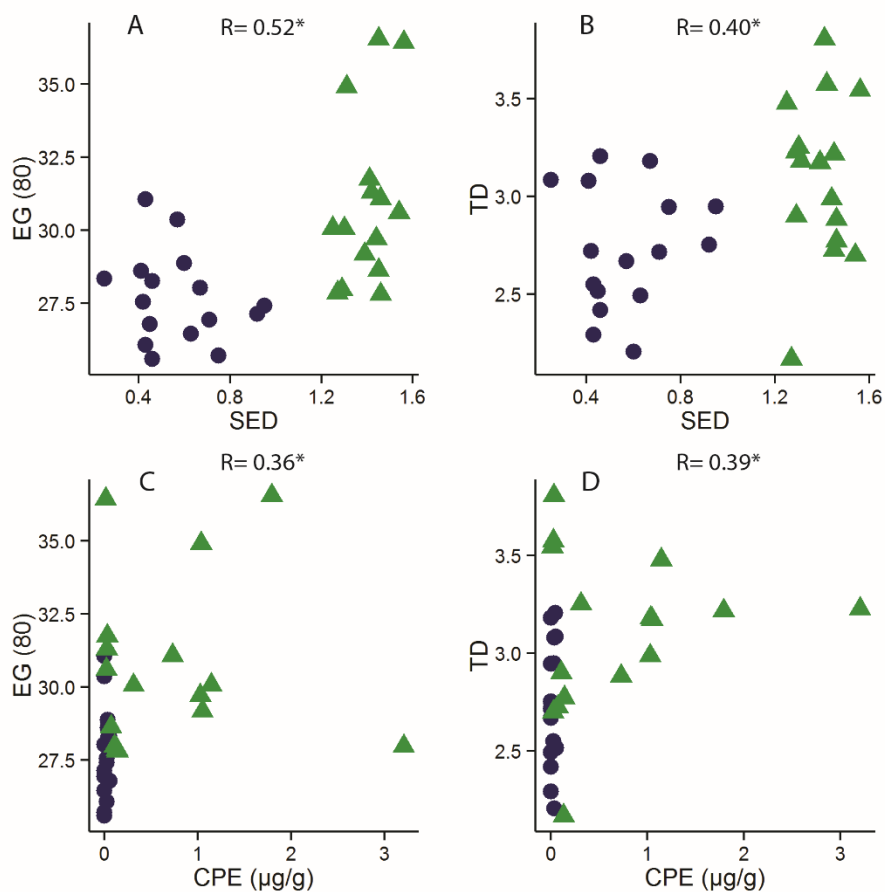



Figure 6. Correlations between (A) Sediment particle-size diversity (SED) and Expected genus diversity (EG (80)), (B) between SED and Trophic diversity (TD), (C) between EG (80) and Chloroplastic Pigment Equivalents (CPE) and (D) between TD and CPE. Green triangles represent correlations for shelf-break stations and dark blue circles between slope stations. R indicate correlation values of Spearman. * shows significant correlations.

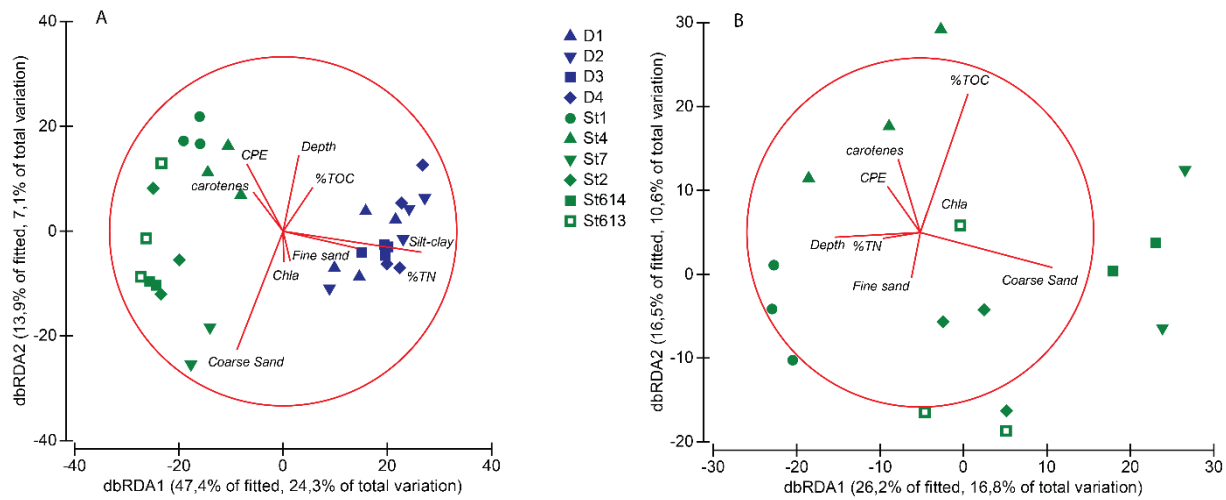



Figure 7. Distance-based redundancy analysis (dbRDA) illustrating the DistLM model based on the genera assemblage data for slope (blue) and shelf-break (green) stations (A) and only between shelf-break stations (B). Fitted environmental variables are shown with their vectors (strength and direction of effect of the variable on the ordination plot).

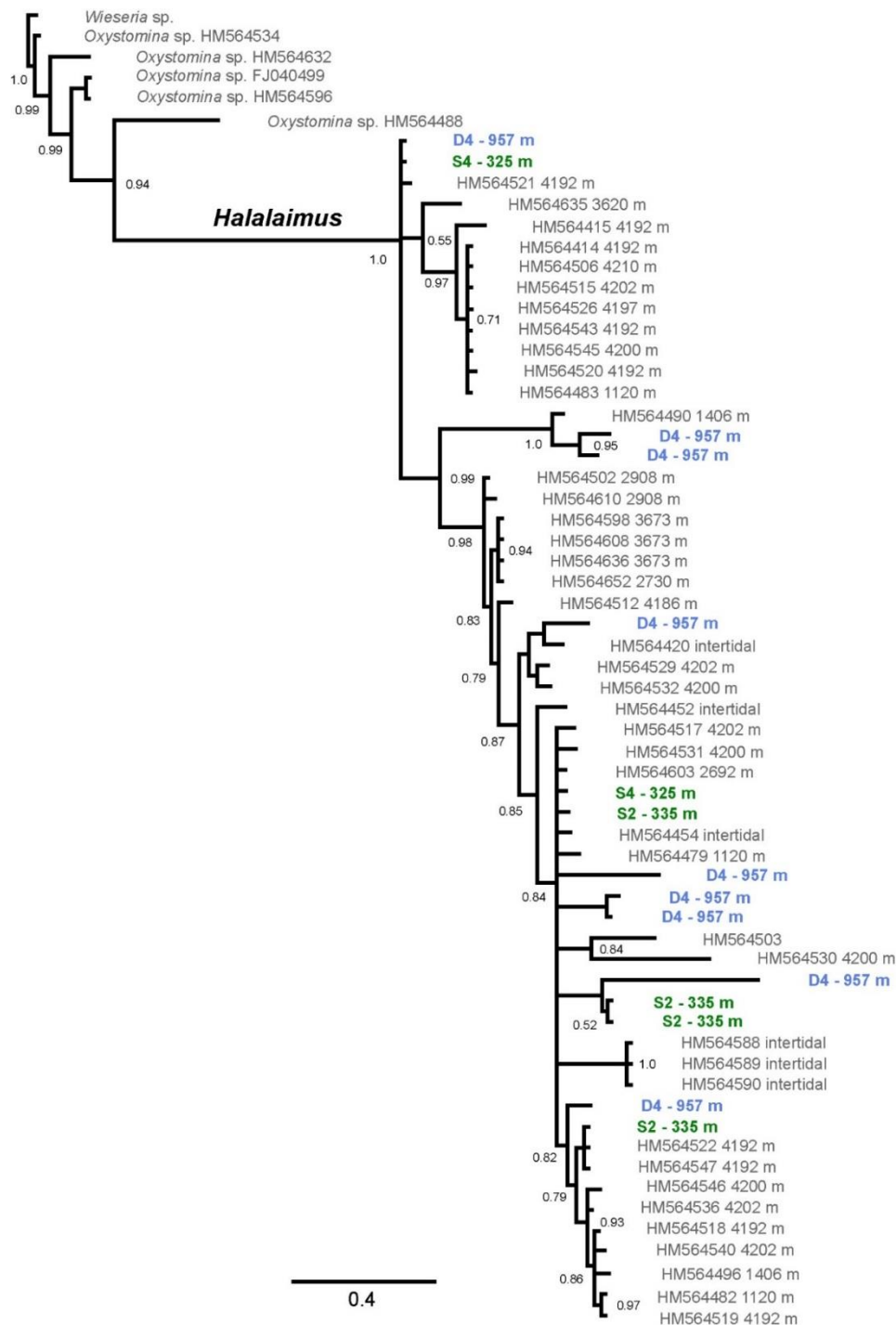



Figure 8. Consensus of Bayesian inference of phylogeny of the genus *Halalaimus* based on 18S rDNA sequence fragments generated in this study and from Bik et al. (2010); node support is given as posterior probabilities (PP);

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.

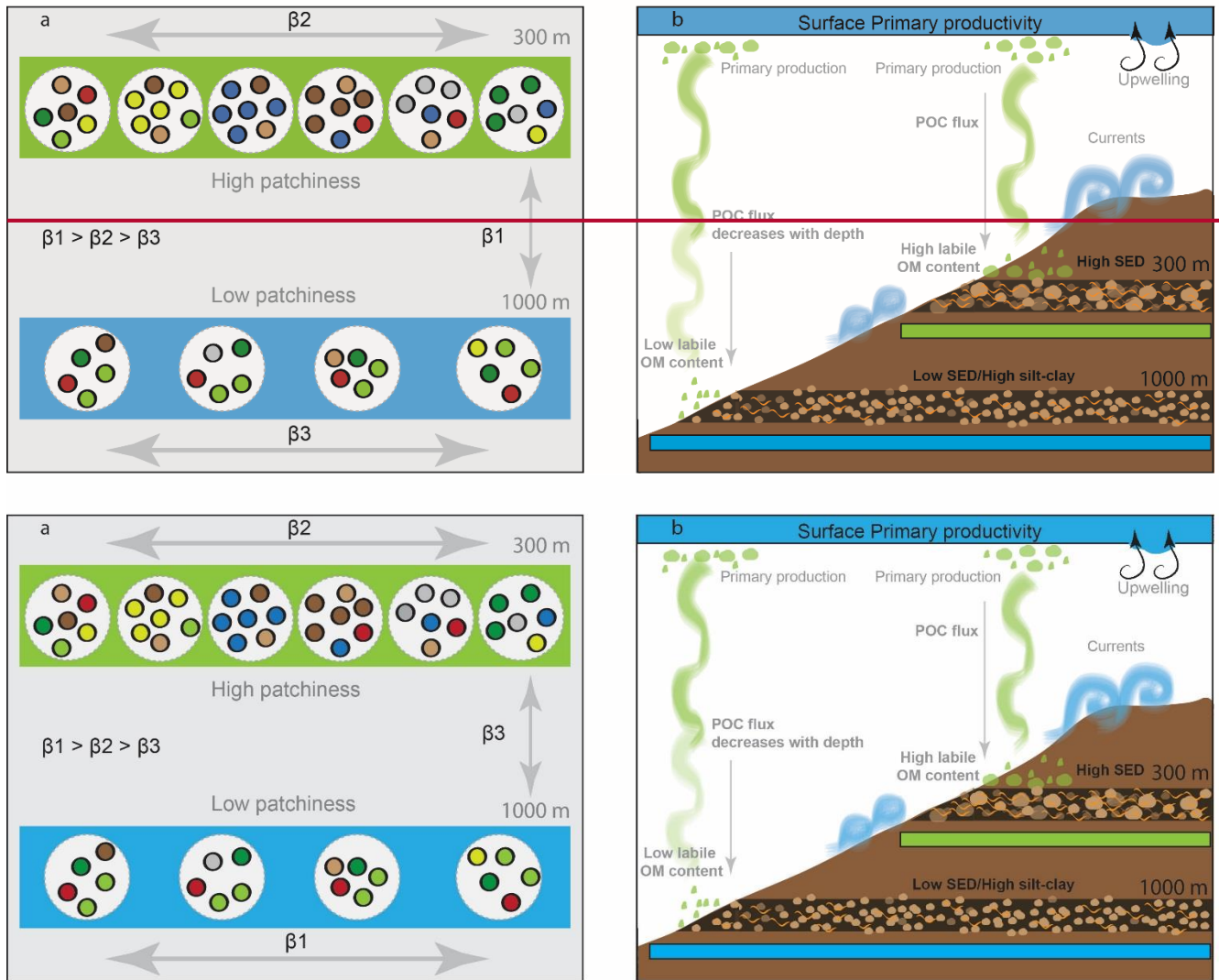



Figure 9. Scheme showing (a) how beta diversity varied across stations and between bathymetrical transects. The green bar represents the 'shallow' transect and the light blue bar the 'deep' transect. Grey circles inside the bars represents the stations sampled at each transect. Coloured circles inside grey circles refer to the variability in nematode genus composition within station and across stations. It illustrates the higher densities and turnover (beta2) ~~patchin~~ found at the 'shallow' stations and the lower turnover (beta 1) found at the 'deep' stations. The (b) figure reveals the main environmental factors assumed to be 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 composition at the 'shallow' transect when compared to the deeper transect. The green bar represents also here the 'shallow' transect and the light blue bar the 'deep' transect.