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Species variability and connectivity in the deep sea: evaluating effects of spatial heterogeneity and hydrodynamics

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

5   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
10 [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)

15 **Abstract**

Understanding processes responsible for shaping biodiversity patterns on continental margins is an important requirement for
comprehending anthropogenic impacts in these environments. Continental margins perform crucial functions which are mainly
structured by hydrodynamic effects and surface primary productivity. However, to what extent these processes control benthic
local and regional biodiversity remains unclear. In this study, we focused on two isobathic parallel transects to test if food
20 resources arriving at the seafloor and hydrodynamic effects affect alpha and beta diversity at different depths in similar ways.
We also examined the potential role of connectivity between both depths as a result of dispersal. This is the first study applying
integrative approaches using molecular and morphological techniques in combination with environmental factors to unravel
spatial variability and connectivity in relation to depth in the deep sea. Results revealed that high variability in resource
availability is directly linked to high alpha diversity and spatial heterogeneity, and that communities dwelling in deeper regions
25 are able to use resources complementarily promoting species coexistence. Our study also demonstrated that higher
hydrodynamics at the shallower habitats near the shelf break, as inferred from the high sediment heterogeneity, promoted
variation in community structure across stations (higher beta diversity) compared to the deeper area. In addition, phylogenetic
relationships revealed no evidence for depth-endemic lineages or isolation per habitat, indicating regular species interchanges
across different depths.

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

1. **Introduction**

The link between biodiversity (i.e. diversity of species) and ecological processes (e.g. carbon flow, species 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 coast and the abyssal plains of the deep sea, continental margins (0–4000 m) encompass the largest habitat diversity in the marine environment (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 many 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 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 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). Probably 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), drive small-scale (1–10 m²) patterns of benthic communities (Gage, 1997) promoting alpha diversity (Levin et al., 2001). Besides, other factors also play a role in shaping benthic communities. High community differences over large spatial scales (100–1000 m²; beta diversity) within continental margins has been observed both along a bathymetric gradient as well as between stations of similar depth. This indicates that beta diversity is not singularly depth-dependent (Danovaro et al., 2013; Havermans et al., 2013). Physical factors, including near-bottom currents, sediment grain-size heterogeneity, boundary constraints, hydrodynamics, 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. In this sense, continental margins are recognized as highly heterogeneous systems (Levin and Dayton, 2009). Most benthic species have restricted active dispersal potential, but passive dispersal may be facilitated through ocean currents, especially for species with pelagic larval stage (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 metazoa 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; Derycke et al., 2005). Nematodes therefore, hold ideal life traits when seeking to understand connectivity, 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 would contribute another explanation for why the bathyal holds such a high biodiversity (Rex and Etter, 2010). While empirical data for macrofaunal molluscs, crustaceans, as well as octocorals has been found in support of 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) and which may be realized by underestimated use of near-bottom currents as dispersal vectors.

In this study, community diversity was assessed along two isobathic parallel transects at the Western Iberian Margin. Potential drivers for turnover in nematode taxonomic composition were analysed at three spatial scales: within stations, between stations from the same depth, and between the two depth transects. To evaluate potential depth-mediated differentiation, connectivity between the two bathymetric zones was investigated based on 18S rDNA sequence data of selected nematode taxa. The following hypotheses were tested: (H1) The patchiness of food resources deposited at the seafloor results in a higher alpha diversity; (H2) Disturbance (high hydrodynamics) increases habitat heterogeneity, resulting in a higher beta diversity; (H3) Beta diversity between different bathymetric transects is higher than beta diversity across similar depths; (H4) There is connectivity between shallow and deep 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

5   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; Salgueiro et al., 2010). The high particle transport observed at the WIM occurs mainly due to the great hydrodynamics 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

10   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, situated

15   294–445 m deep (further referred to as shallow transect), just beyond the shelf break; the second transect was located at the mid-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

20   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 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., 2012). Total sedimentary organic carbon (% TOC) and nitrogen (% TN) were determined with a Carlo Erba

25   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 (Chl$a$), 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

30   equivalents (CPE: Chl$a$ + 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 glycerin (De Grisse, 1969), mounted on glass slides and identified to genus level using relevant literature (Vanaverbeke et al., 2015; Warwick, 1998).

5   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):

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

10   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, Chl$a$, CPE, carotenes, depth, sediment grain size and
15   SED) and univariate nematode variables (H', J', EG (80), and TD) were investigated by means of Spearman rank correlations and Draftsman plots (Anderson, 2007) in R (R Core Team, 2013).

The nematode community data on genus level were analysed based on Bray-Curtis similarities (and Euclidean distances for the univariate data) by means of non-parametric multivariate ANOVA (PERMANOVA; Anderson, 2007) to assess differences between 'deep' and 'shallow' areas (2-factor nested design) and between all stations across both transects
20   (1-factor design). The 2-factor model included 'depth' as a fixed factor and 'station' as a random factor nested in 'depth'. The 1-factor model design used 'station' as a fixed factor. 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, 2007). Subsequent pairwise 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
25   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.

The multivariate environmental data was first normalized (subtracted mean divided by standard deviation) and
30   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 (Chl$a$, 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.

5   2.5.   Molecular phylogenetic analyses of nematodes

         One sample 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,
10 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
15 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.

20   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. 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
25 reverse sequences) generated during this study were aligned using the MAFFT 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
30 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 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.

The *p*-distances for each genus were calculated in MEGA 6.0 using pairwise comparisons and pairwise deletion of gaps.

3. Results

3.1. Environmental parameters

Biogeochemical and granulometric properties of the sediment are shown in Figure 2. Sediment particle-size diversity (SED) significantly decreased ($p < 0.05$) at the 'deep' transect. The sediment composition at the 'deep' stations was mainly composed of silt-clay fractions (81–89 %), while at the 'shallow' stations fine sand (25–42 %) dominated, except for S7, where medium sand showed a higher proportion (30 %). Nested PERMANOVA results showed significant differences 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 (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 station for these three variables (Fig. 2). Chl*a* (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. Chl*a* 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 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

5 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 *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

10 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 '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

15 (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 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],

20 [S1, S614], [S7, S2] and [S2, S614].

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. In general, dissimilarity values between stations increased with increasing depth and geographical distance (Fig 5). SIMPER analysis revealed that the genera *Acantholaimus, Microlaimus*, *Richtersia,* and

25 *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 constant 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,

30 S7] and [S7, S2] possessed significant differences (p < 0.05) in nematode genera composition. When PERMANOVA tests (one-way PERMANOVA) were calculated between all stations, 'deep' stations significantly differed from 'shallow' stations (p < 0.05) but the pairs [D1, S1], [D1, S4], [D2, S4], [D4, S1], and [D4, S4] were not significantly different from each other (p > 0.05).

Trophic diversity revealed significantly higher values at the shallow stations (p < 0.05) but no significant pairwise comparison (p > 0.05). 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, Chl*a*, 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 Chl*a*, 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). Considerable higher silt-clay content was observed in the 'deep' stations. 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

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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

5 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**

4.1. (H1) The patchiness of food resources deposited at the seafloor results in a higher alpha diversity

10 In this study, the high variability of food distribution within shallow stations (Fig. 2) observed at the Western Iberian Margin (WIM) was positively associated with a high alpha diversity in terms of trophic group composition and genus community diversity (Fig. 4). The export flux and the distribution of labile organic matter on the seabed are related to the high patchiness observed in surface primary productivity (Crespo et al., 2011). As such, they are resulting from the interactions between coastline features and wind forcing, as previous studies stated (Crespo et al., 2011). In this regard, surface processes

15 dynamic changes over time and space will also affect resource distribution (Cardinale et al., 2000). Our data can hence be connected to productivity-related processes, such as upwelling events, pulsed food export, and quality of organic matter, which are thought to indirectly affect species richness (alpha diversity). These processes act as surrogate factors of small-scale patchiness and habitat heterogeneity, shaping patterns of species diversity (Tokeshi, 1999). This idea is supported by our observed pattern of a decrease of 'labile' organic matter with an increase in depth (Fig. 2) that was also related to a lower

20 variability of resources within and between deep stations, as well as a decline of alpha diversity (Fig. 4). A similar relationship between decreasing availability of 'labile' organic matter associated to a decrease in diversity was also observed by Leduc et al. (2012) for another slope at similar depths and may thus represent a general pattern at continental margins.

The generally low food input in the deep sea might favour organisms adapted to thrive in this food-deprived environment. The deposit-feeding behaviour of *Halalaimus* in this study may explain its success and reflect its high fitness in

25 the deep sea in general (Fig. 3), because deposit feeding can be interpreted as an adaptation to the low food input. 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 presence of the epistratum feeder *Acantholaimus* in higher abundances at the deeper transect was expected (Fig. 3), since this genus is considered a typical deep-sea genus. *Acantholaimus* is increasing in abundance from the shelf break towards abyssal plains and dominating nematode communities

30 at all these depths (Vanreusel et al., 2010). Up to 51 species of *Acantholaimus* were already described (Miljutin and Miljutina, 2016) and a large number of different species can coexist (Muthumbi et al., 2011). By possessing a large variation in its mouth parts, *Acantholaimus* coexistence indicates a possible food resource partitioning (Muthumbi et al., 2011). However, whether

the restriction to deep regions is solely food-related or whether other abiotic/biotic factors play a role in the distribution of *Acantholaimus* is still not clear.

A general dependency on the surface-derived organic matter arriving at the seabed has already been observed for all benthic size classes (macro-, mega-, and meiofauna) (Amaro et al., 2009; Lins et al., 2015; Veit-Köhler et al., 2013; Würzberg
5 et al., 2011). In addition, changes in feeding habits according to the food input rate were detected across different taxonomic groups of the benthos (Amaro et al., 2009; Moens and Vincx, 1997). Our findings support this change in feeding mode, as shown by our data on the trophic composition, associated with the depth-dependent decrease in food supply. This means that, when 'fresh' organic matter is scarce, feeding may generally rely on refractory organic material or even in facultative predatory behaviour (Fonseca and Gallucci, 2008). A similar trend was reported for the nematode genus *Pontonema,* which showed
10 facultative predatory behaviour in the deep sea, but not in shallow water as a consequence of food deprivation in the first (Fonseca and Gallucci, 2008). These strategies enable organisms to cope with various changes in food availability and suggest a highly adaptive behaviour of deep-sea organisms, including nematodes, and the tendency to use resources complementarily (Cardinale et al., 2000; Moens and Vincx, 1997).

15 4.2. (H2) Disturbance (high hydrodynamics) increases habitat heterogeneity, resulting in a higher beta diversity

Distance-based linear models (DistLM) displayed a major significant effect of the silt-clay proportion on community variation when compared amongst distinct depth transects (Fig. 7). Higher variation in sediment composition together with an increase in sediment particle-size diversity (SED) at the 'shallow' stations were most probably related to strong near bottom current pulses already reported at the WIM (Quaresma et al., 2007). Here, the increase in sediment and nematode community
20 dissimilarity with increasing geographical distance and depth (Fig. 5) indicate a rapid species turnover at the WIM. Moreover, the higher dissimilarity for both nematode community and sediment at the 'shallow' transect (Fig. 5) suggest higher hydrodynamics in this area. Near-bottom currents at the WIM, varying from 0.1–0.2 m s^{-1}, have the capacity to erode and suspend sediment (Drago et al., 1998; Quaresma et al., 2007). In addition, it has been asserted that bottom flow at the WIM may cause high energetic conditions, resulting in areas rich in coarse sand and poor in silt-clay, which is more easily maintained
25 in suspension (Quaresma et al., 2007). Furthermore, the WIM is known to be subjected to fisheries, applying bottom trawls down to a depth of 800 m water depth, which can also affect the continuous mixing and resuspension of surface sediments from the continental slope (ICES, 2008; Pusceddu et al., 2014). Consequently, if higher resuspension rates of sediment can occur, especially at depths shallower than 800 m, this will also affect resuspension of organisms dwelling at or within the sea bottom while increasing variability in relation to more stable environments (e.g. the deep transect).
30 Our study demonstrated higher trophic diversity (TD) and relative abundances of deposit feeders in the deep stations, together with a higher hydrodynamic stability, also 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 also observed by Leduc et al. (2012) for similar depths, although here SED and TD values reported, were much higher. Trophic diversity at the shallow

stations showed greater variation (Fig. 4), illustrating higher niche differentiation across stations, reflecting the possible transient stage of this habitat. Moreover, higher prominence of opportunistic species and fast colonizers (epistratum feeders), such as *Microlaimus*, was observed at shallow stations in this study (Fig. 3), which were further characterized by higher food input as shown by the greater Chl*a* values. The dominance of opportunists in waters below 200 m (Muthumbi et al., 2011; Pape et al., 2013; Raes et al., 2010) has been observed in many deep-sea areas in association with disturbance events, where communities are dominated by few species during the entire recolonization process (Lee et al., 2001). The observed high densities of *Microlaimus*, which is 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 relatively strong hydrodynamics and possible anthropogenic disturbance effects from fisheries at the WIM, which influence the bottom dynamics at the shallower stations. Disturbance effects via either bedload movement or erosion and sedimentation of suspended load alters not only particle size, but also organic content. Effects on the benthic fauna are mainly observed on deposit feeders, with an increase in deposit feeding during transitional and depositional phases, when hydrodynamics or other disturbance effects decrease (Gage, 1997).

At the WIM the strong non-linear waves corroborate the idea of small-scale disturbance and possible colonization by organisms more suitably adapted to stress conditions. Thus, the higher alpha and beta diversity observed at the shallow stations (mainly represented by low occurrence genera) indicates that disturbance levels in this area enhance not only alpha but also beta diversity. The WIM is characterized by the alternation between strong and weak winds, which in turn drive benthic storms and upwelling processes, and consequently determines the behaviour of benthic organisms (Vitorino et al., 2002). It can be assumed that whenever conditions are more favourable, such as during weak bottom-current periods, rare genera, for instance those with long life cycles, could become more important in terms of abundance and biomass (Bongers et al., 1991). In this sense, environmental fluctuations determine the identity of the dominant competitors, which are in our study *Halalaimus* in the deep and *Microlaimus* at the shallow stations. They also provide opportunities for other species to establish (Fox, 2013). As a consequence, the observed species diversity will be a trade-off between the frequency of disturbance, providing opportunities for new species to overcome competitive exclusion, and ecosystem stability, resulting in an increase in habitat heterogeneity (Gage, 1997).

4.3. (H3) Beta diversity between different bathymetric transects is higher than beta diversity across similar depths

Sediment grain-size characteristics, an indicator for hydrodynamics, were clearly more heterogeneous between shallow stations when compared to deep stations (Fig. 2). Differences in hydrodynamics promote habitat heterogeneity and consequently beta diversity (Gage, 1997). Variation in near-bottom currents were hence probably the main driver responsible for among-station variation within the shallow transect. Contrastingly, the high silt-clay contribution together with the low variation within and between stations observed at the deep stations (Fig. 2) was associated with the lower beta diversity in this area (Fig. 4). As mentioned earlier, the stability of this environment, in opposition to a higher expected genus diversity (EG (80)) and TD at the shallow stations, favoured the dominance of the genera *Acantholaimus* and *Halalaimus*, commonly abundant in relatively undisturbed deep-sea soft sediments (Vanreusel et al., 2010).

In general, dissimilarity in genus composition increased with both depth and geographical distance (Fig. 5). Additionally in this investigation, beta diversity between depth transects was higher than within transects, although distances between stations and transects were comparable. Other studies 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). Here, the strong differences in sediment composition and food resource patchiness between transects (Fig. 2) were likely the main factors responsible for the higher beta diversity observed between the two transects. These findings suggest that diversity changes can be associated with both large and small-scale features. 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).

While sediment grain size did not show strong variability among cores from the same station (Fig. 2), high among-core variation in the density of the most abundant genera was observed (Fig. 3). Both these most abundant genera, as well as the presence of low occurrence genera in each core implies that within-station turnover is also affected by small-scale patchiness, such as different availability, composition and size spectra of food particles (Danovaro et al., 2013), as it was shown by the increase in EG (80) and TD with increasing 'labile' organic matter (Fig. 6). In this study, however, food resource distribution alone, as shown by the trophic composition, does not explain the high within-station variability in diversity observed at the shallow transect. Additional ecological factors and biotic interactions not measured here could account for the high alpha diversity observed at the WIM (> 40 % within station variation), such as competition, predation, dispersal dynamics, and patch extinction (McClain and Barry, 2010). Nevertheless, when predation levels are high, the proportion of juveniles in a community should also be high, due to constant predation and "cropping" of standing stocks (Grassle and Sanders, 1973) or due to the unsuitability of juveniles in a predator diet caused by their smaller size (dos Santos and Moens, 2011). In our study, the juvenile/adult proportion varied from 0.8 to 1.0, indicating no differences in abundance between these groups, and may indicate possibly weak predation effects (McClain and Schlacher, 2015).

Human-induced activities, such as bottom trawling and drilling activities, were not measured in this study, but should be considered as a potential factors causing disturbance and impacting habitat heterogeneity, especially at the shallow stations studied here. Trawling activities are very common at the WIM and negative impacts on sediment resuspension and benthos mortality were reported for other slopes, as well as a decrease in benthos abundance and species richness (Pusceddu et al., 2014; Sparks-McConkey and Watling, 2001).

4.4. (H4) There is connectivity between shallow and deep

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 be indicative of connectivity between the two (Fig. 3). In our study, phylogenetic relationships within and between the genera sampled at both shallow and deep stations potentially revealed shared species and thus connectivity across depth. 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; 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 and Bower, 2015; Etter et al., 2011; Etter et al., 2005; 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 and Bower, 2015; Etter et al., 2011). 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 connectivity across depth, suggesting taxon-specific barriers (Bik et al., 2010; Riehl and Kaiser, 2012).

In our study, phylogenetic relationships within and between the genera sampled at both shallow and deep stations did not reveal any evidence for depth-endemic lineages or isolation Fig. S1). Instead, shallow and deep specimens were intermingled in the phylogenetic reconstruction. This result indicates frequent exchange and connectivity between bathymetrically different habitats. 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 in this study 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, connectivity 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 connectivity between shallow and deep habitats, other studies have suggested endemism in deep-sea habitats (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).

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 CO1 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

5   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

10 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 high dispersal (gene flow), while other species may have limited dispersal abilities.

5. Conclusions

Our results reported a high variability of resource distribution (Chl*a*, CPE, carotenes) and sediment composition

15 within and between the shallow stations, associated with a high alpha and beta diversity at the WIM (Fig. 9). High alpha diversity was mainly related to a complementary use of food resources whereas higher hydrodynamics at shallower habitats (inferred by the high sediment heterogeneity) promoted greater variation in community structure across stations (higher beta diversity) compared to deeper areas, favouring organisms tolerant to disturbance to become more abundant. Coarser sediment composition at the shallow stations indicated that strong near-bottom current pulses can have positive effects on the benthic

20 fauna, promoting diversity through the creation of suitable patches. The higher beta diversity observed across transects highlights the increase in stability and dominance of particular genera with increasing depth. To conclude, phylogenetic relationships suggested connectivity between deep and shallow waters for the nematode genus *Halalaimus*.

6. Acknowledgements

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7.   References

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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

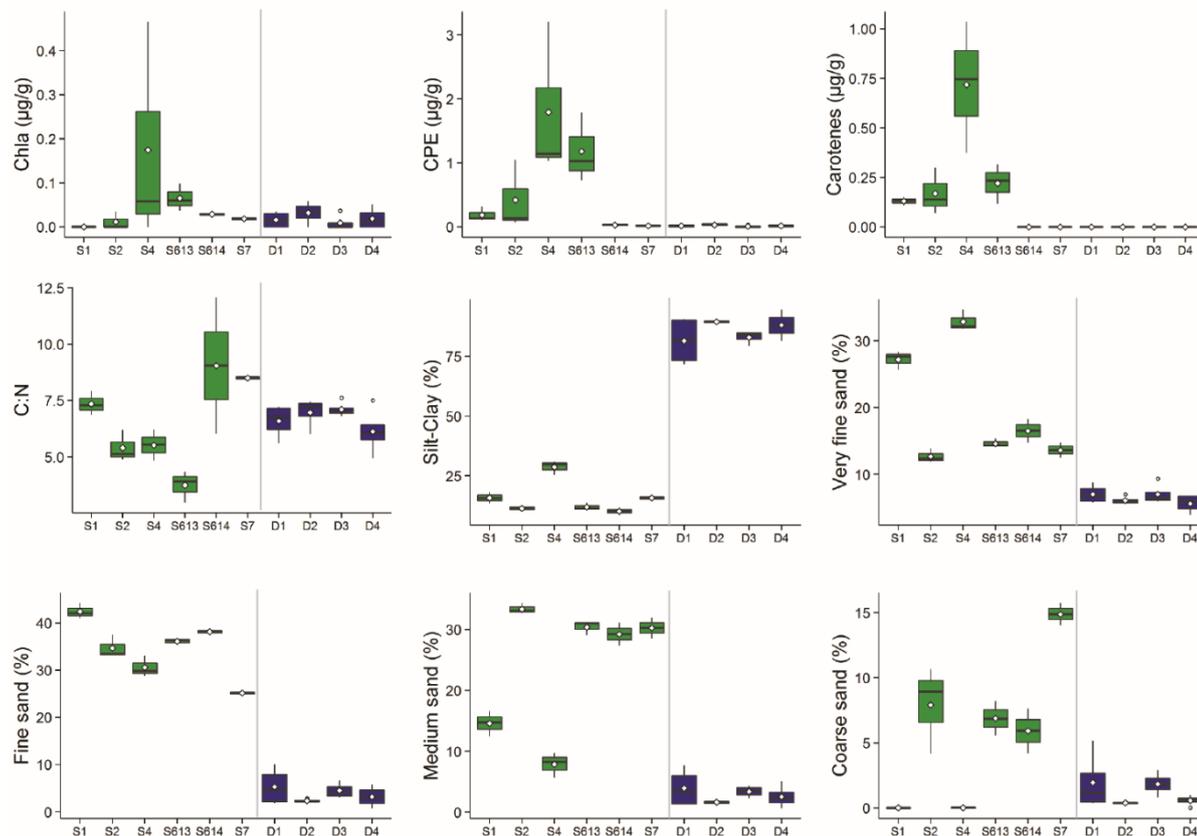| deep4	6/06/2014	987	37°49'375"N	09°27'839"W

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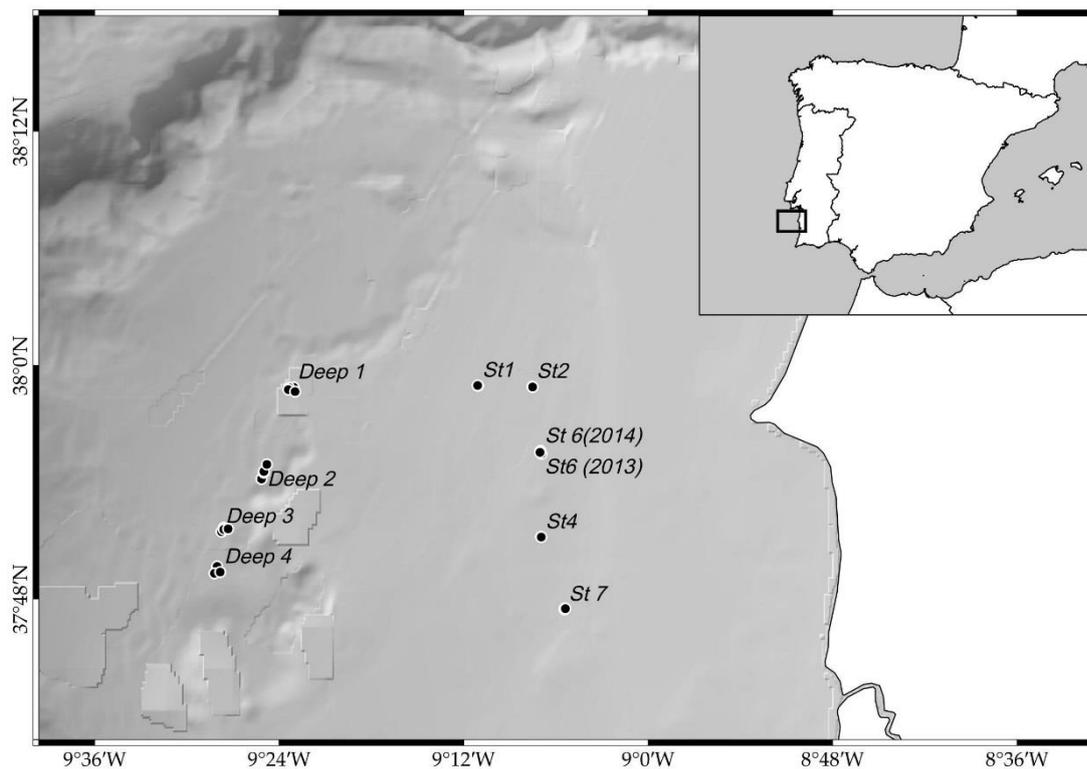
Figure 1. Location of B2013/17 and B2014/15 stations. Sampling stations are represented by black circles.

Figure 2. Environmental variables used in this study per station: Chl*a* (Chlorophyll a), CPE (Chloroplastic Pigment Equivalent), Carotenes, C/N (ratio between % TOC/% TN), 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.
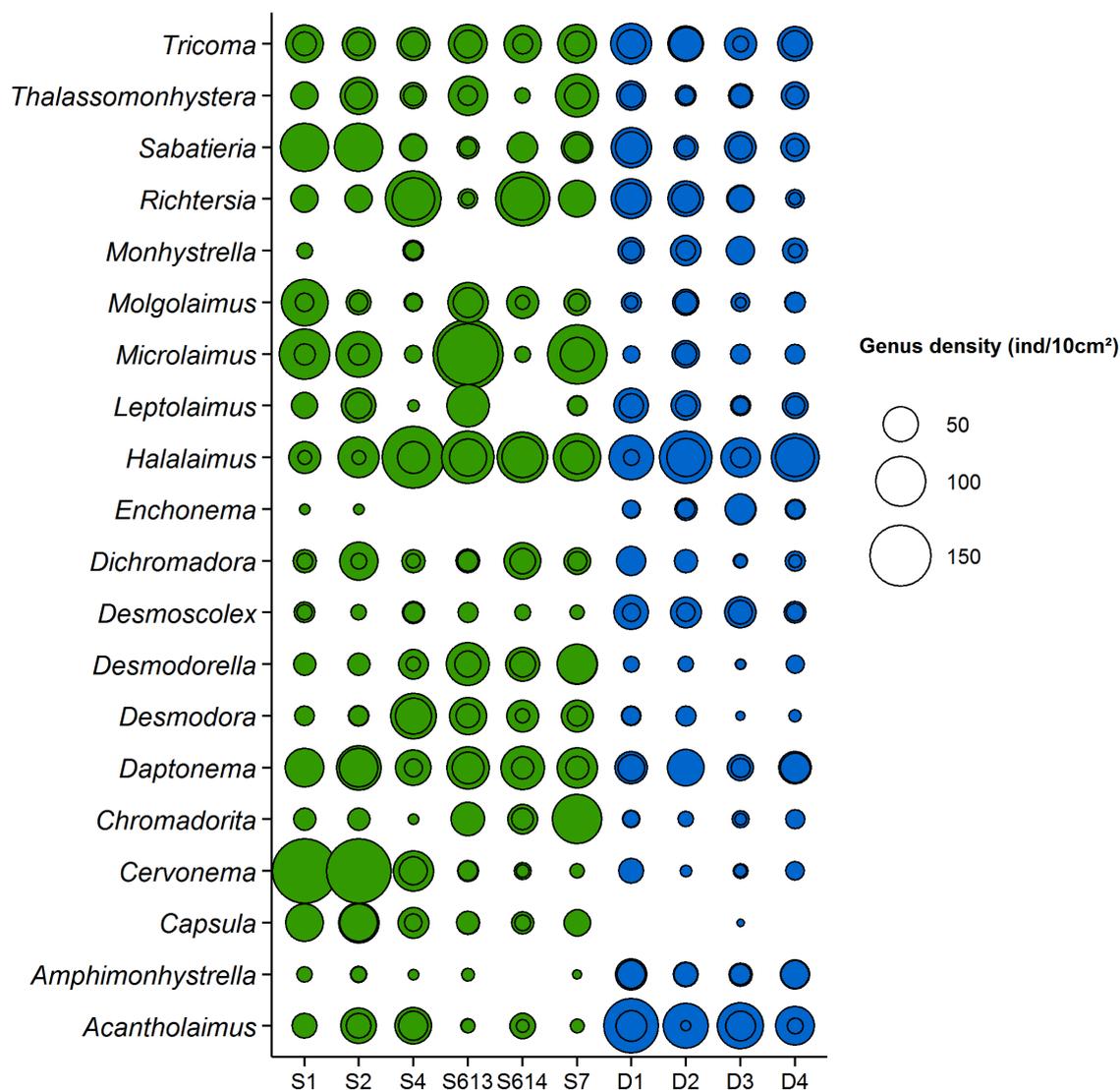
Figure 3. Most abundant nematode genera (> 4 %) per station. Inner circles indicate genus density (10 cm²) and outer circles genus standard deviation. Green circles represent shallow stations and blue circles deep stations.
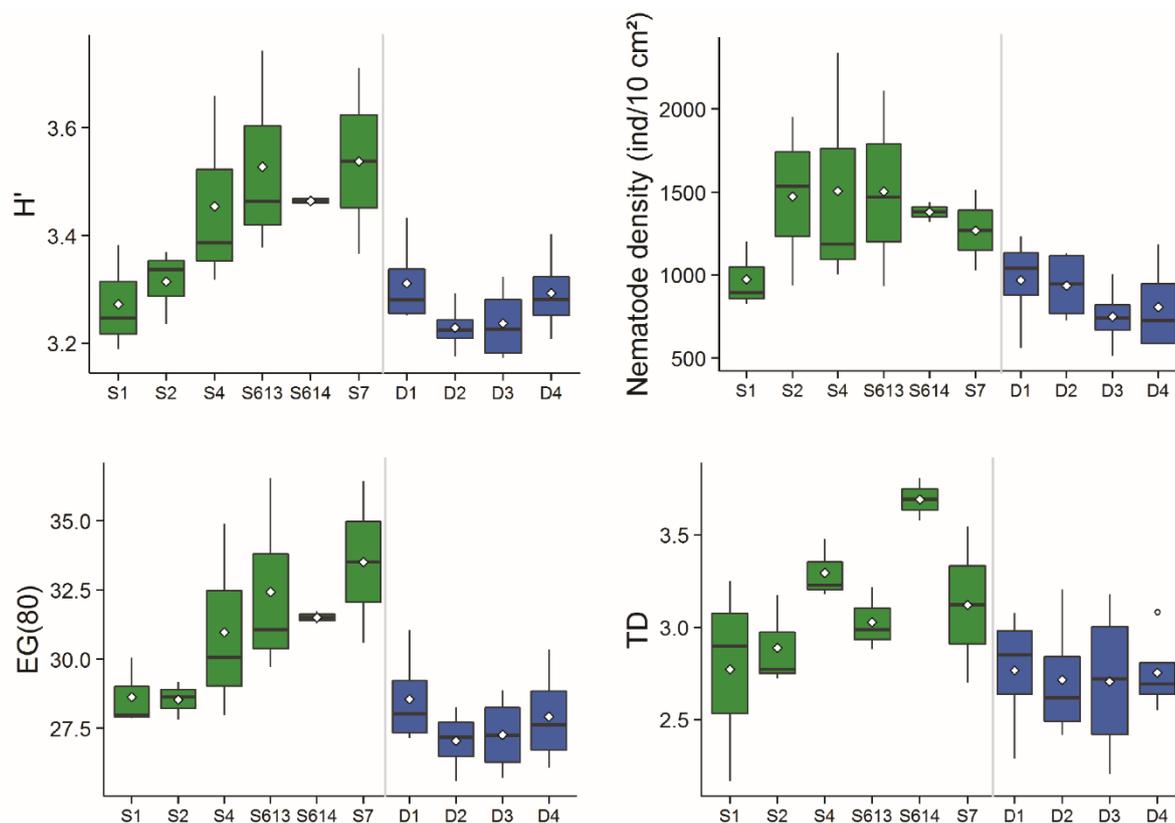
Figure 4. Nematode Shannon-Wiener (H') diversity, nematode density (individuals/10 cm²), Expected number of genera (EG(80)) and trophic diversity (TD) per station. Green boxplots represent shallow stations and blue boxplots represent deep stations. Black lines represent the median, empty circles represent 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.
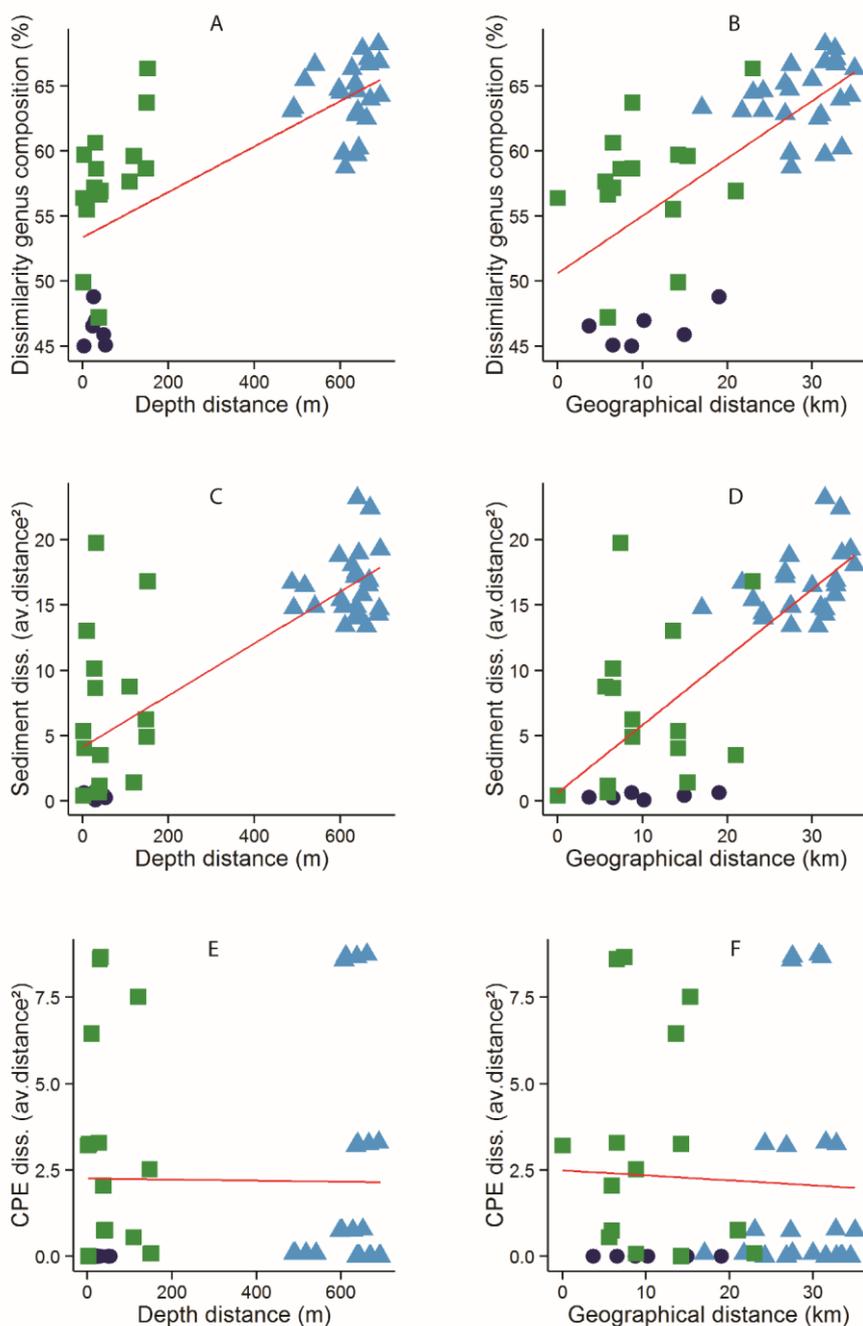
Figure 5. Dissimilarity values in nematode genus composition among depth difference (A) and between geographical areas (B), in sediment composition among depth difference (C) and between geographical areas (D), and in chloroplastic pigment equivalents (CPE) among depth difference (E) and geographical 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.
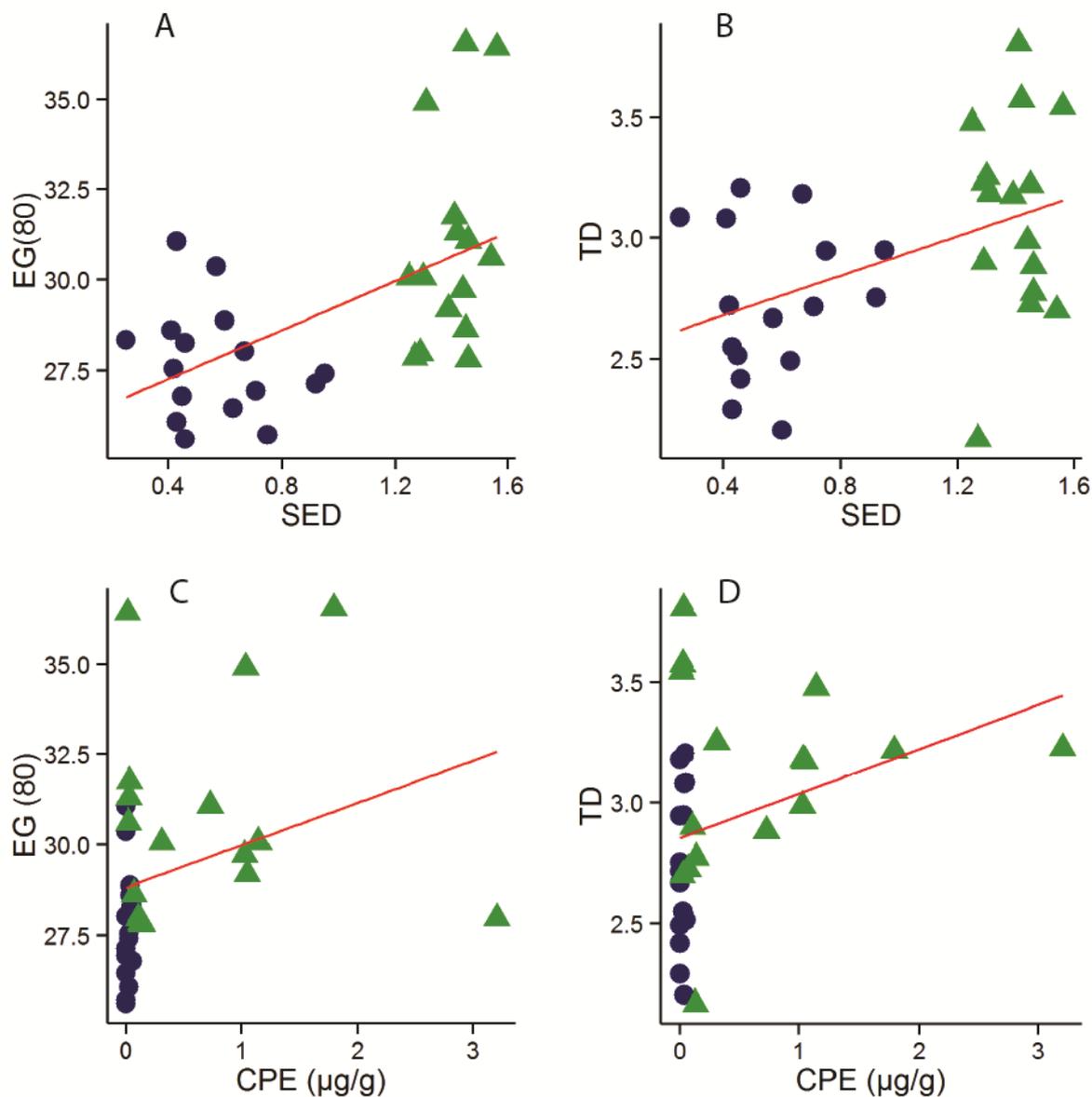
Figure 6. Correlations between (A) Sediment particle-size diversity (SED) and Expected genus diversity (EG (80)), (B) between SED and Trophic diversity (TD), (C) between EG (80) and Chloroplastic Pigment Equivalents (CPE) and (D) between TD and CPE. Green triangles represent correlations for shallow stations and dark blue circles between deep stations. Red line indicate linear trends.
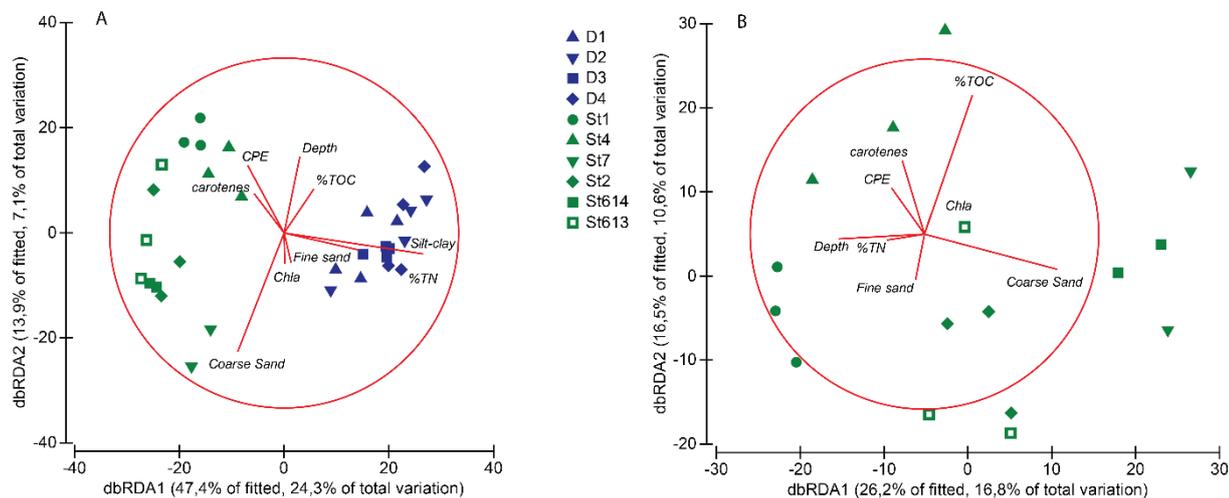
Figure 7. Distance-based redundancy analysis (dbRDA) illustrating the DistLM model based on the genera assemblage data for "deep" (blue) and "shallow" (green) stations (A) and only between shallow stations (B). Fitted environmental variables are shown with their vectors (strength and direction of effect of the variable on the ordination plot).
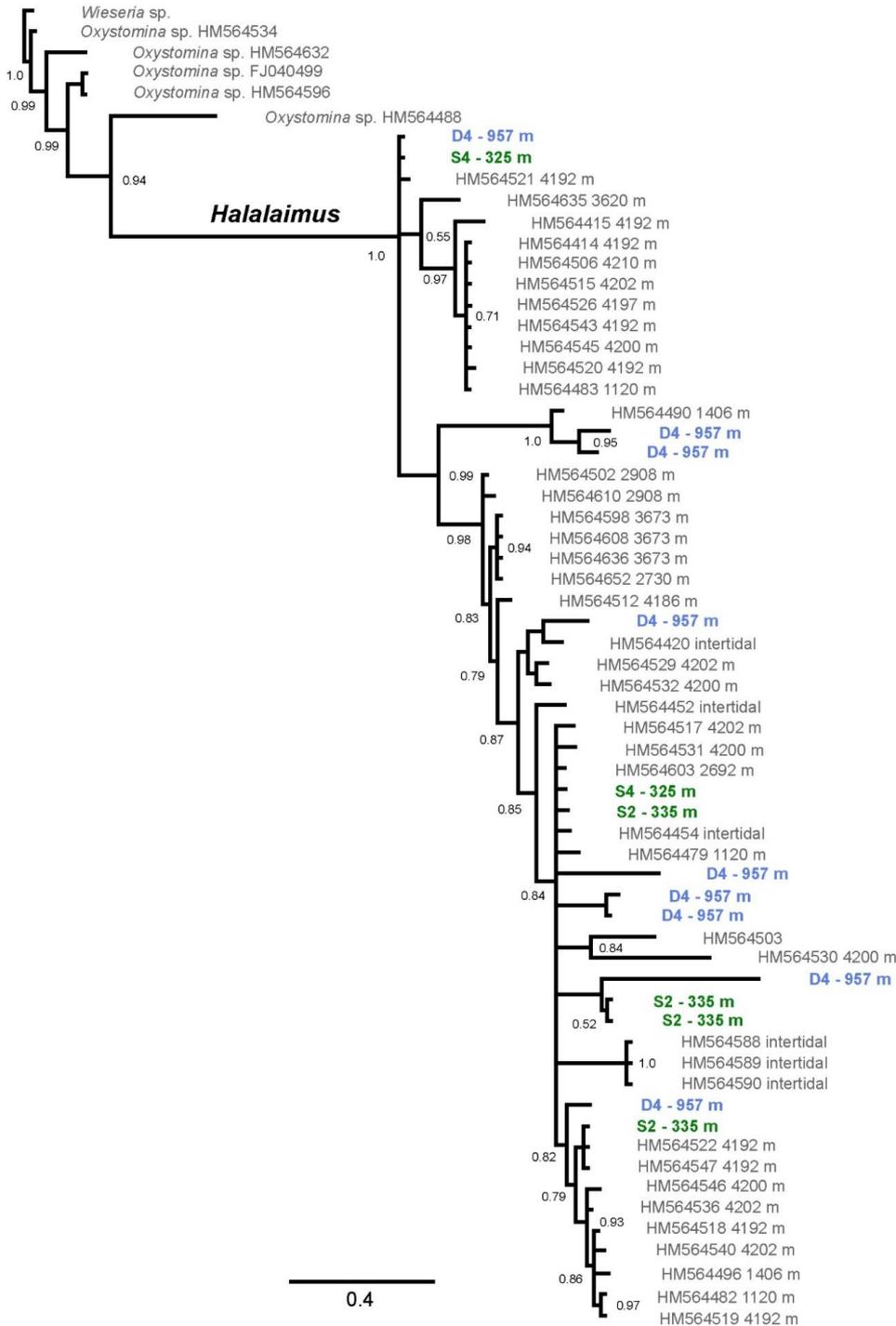
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Figure 8. Consensus of Bayesian inference of phylogeny of the genus *Halalaimus* based on 18S rDNA sequence fragments generated in this study and from Bik et al. (2010); node support is given as posterior probabilities (PP); nodes with PP smaller than 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.
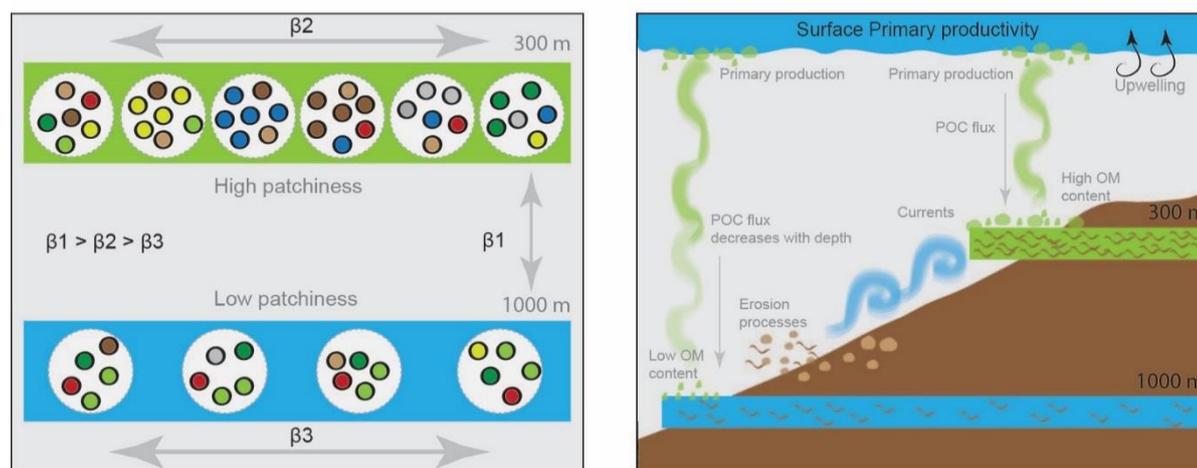
Figure 9. Scheme showing (left) how beta diversity varied across stations and between bathymetrical transects, and the higher patchiness found at the 'shallow' stations, and (right) the main environmental factors responsible for beta diversity between both depth transects. Upwelling effects, primary production, currents, disturbance causing erosion processes, and decrease of POC (particulate organic carbon) with increasing depth are considered to be correlated with both alpha and beta 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. Moreover, erosion processes are illustrated through the resuspension of sediment and organisms (e.g. nematodes) into the water column as a consequence of strong hydrodynamics.