Dear Editor Marilaure Grégoire,

We have addressed all the critics and suggestions of the two reviewers in this document, as well as the concerns reported by the editor. We have deepened specifically our response to reviewer #2 concerning the use of replicates and added to the introduction of our text and throughout the text the importance of our work.

We want to emphasize that this is the first time in ecological studies in the deep sea that comparisons between two isobathic transects along the slope is performed. By using this sampling design, one can test whether environmental variables are as important or more important than spatial differences in defining community distribution and coexistence. In general, most of the studies are restricted to a bathymetrical gradient, making it difficult to compare whether depth or the variation in environmental patterns linked to the increase of depth is the main factor causing differences in benthic communities.

Finally, we would like to thank the editor for the support and kindness in scientific communication.

Kind Regards,
Lidia Lins, on behalf of all authors

Letter to reviewer #1:

Dear Anonymous Referee #1,

Thank you very much for your valuable comments. We have revised the manuscript and have included most of your suggestions.

We have addressed each of your comment below:

1)'P3, L12: The authors state that "nematodes may be passively transported via water currents following resuspension from disturbance events". Is there any information available on how rapidly they are likely to re-settle? The potential for dispersal will be very different depending on whether they sink passively, actively swim back to the seabed or actively swim into the water column (e.g. Palmer (1984) Invertebrate drift: Behavioral experiments with intertidal meiobenthos , Marine Behaviour and Physiology, 10:3, 235-253, DOI: 10.1080/10236248409378620).'

There is no current information about the exact speed which nematodes re-settle (Ullberg and Olaffson, 2003: Marine Ecology-Progress Series 260, 141-149), but we believe that nematodes are able to choose a spot of preference by using chemical receptors (Choe et al., 2012). Currently there is no study in the deep sea which dealt with the topic, but we believe that nematodes can be carried away with currents and are able to survive if conditions are favourable. We have included a paragraph about resuspension and settlement of particles and how nematodes would behave in relation to sediment resuspension: '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).'

2)'There is relevant theory on cross-slope transport in upwelling systems that would provide further insight into the potential for transport of nematodes between the transects. For example, Condie and Sherwood 2006 (Sediment distribution and transport across the continental shelf and slope under idealized wind forcing. Progress In Oceanography 70(2):255-270) derive length scales for cross-slope transport (in terms of winds and settling rate) that would suggest how many resuspension events would be required to move nematodes between the two transects.'

Thank you for this suggestion. We were not aware of the paper from Condie and Sherwood, 2006 and it was very useful. Based on the average resuspension rate of sediments, settling speeds, and alongshore average transport velocity, we were able to estimate that nematodes would need only 34 h to move from one transect to the other. This result was really interesting. However, we had to shorten the section about possible effects of hydrodynamics on nematodes in the paper because another reviewer did not agree with the section, since we have not directly measured the effect of bottom dynamics.

3) 'The authors can fairly easily explore physical exchanges between their two transects in the context of hydrodynamic model particle transport using the online tool www.csiro.au/connie/, which covers the WIM region'.

The Connie3 tool seem a very useful tool for the WIM areas (even if only data until 2007 can be used). Nevertheless, it appears that the website is not functioning normally, since we have tried many times to extract data unsuccessfully. We have also written to the support email available at the website but no response was provided. In this sense, the use of this tool was not viable for this study.

4)'The language used in relation to the hydrodynamics is unusual and sometimes difficult to interpret. For example: Abstract: Presumably "higher hydrodynamics" refers to stronger or more variable bottom currents or bottom stress. P4, L7: "great hydrodynamics" has no meaning. Perhaps "energetic currents". P11, L15: "4.2. (H2) Disturbance (high hydrodynamics) increases habitat heterogeneity"; again "high hydrodynamics" has no obvious meaning. P11, L30: "Our study demonstrated ..... together with a higher hydrodynamic stability"; again higher is not the right word, and this aspect is not actually demonstrated, but only inferred from the sediment characteristics.'

We agree with the suggestions of the reviewer. Changes were performed accordingly throughout the whole manuscript.

Yours sincerely,
Lidia Lins, on behalf of all authors

Letter to reviewer #2

Dear Anonymous referee #2,

We thank you for your very useful comments and suggestions. We have taken them into account and tried to address them as detailed as possible. We have also included in the manuscript the relevance of our work, as it as a concern of the reviewer. We want to emphasize that this is the first time in ecological studies in the deep-sea shelf break and upper slope (that we are aware) that comparisons between two isobathic transects along the slope is performed. By using this sampling design, one can test whether environmental variables are as important or more important than spatial differences in defining community distribution and coexistence. In general, most of the studies are restricted to a bathymetrical gradient, making it difficult to compare whether depth or the variation in environmental patterns linked to the increase of depth is the main factor causing differences in benthic communities. Through the comparison of different isobathic transects, we could evaluate three different betas: beta diversity within a station, beta diversity between stations, beta diversity between transects. Using this design we expect that, if environmental variables are the main driver of a community, then beta between transects is going to be stronger than beta between stations, since larger environmental differences are expected with increasing depth. Contrastingly, if beta between stations is higher, than spatial differences would play a major role in defining community distribution.

You can find our answers below for each mentioned comment:

1) *"The title should be changed, at present it is far too general. The topic of the manuscript is free-living nematode species variability and connectivity- not species variability and connectivity.*

The reviewer is right. We have changed the title accordingly to: Evaluating environmental drivers of spatial variability in free-living nematode assemblages along the Portuguese margin.

2) *"Moreover, the manuscript does not study effects of hydrodynamics as no hydrodynamic variables are measured. There are a sufficient number of ways that sediment heterogeneity may be obtained that "high" hydrodynamics can not be inferred. I'm also not sure what "high" hydrodynamics means."*

Indeed. The reviewer is right. We have rewritten this section in the discussion. We have not measured hydrodynamics in the area but we have based our assumption of hydrodynamics on previously available data for the Western Iberian Margin to suggest that in our case, sediment heterogeneity could be an indirect measure of hydrodynamic effects. We do not suggest the blind use of sediment as the single measure for hydrodynamics, but rather a combination of those with other data available in the literature. For the WIM region, specifically, because it is characterized by a steep slope, alongshore transport of sediments can be very strong (Condie and Sherwood, 2006; Drago et al., 1998; Quaresma et al, 2007). In our study area, distances between the shallow and the deep station were only ~30 km, indicating that such big changes in sediment composition could be mainly derived from bottom stress derived from alongshore transport of sediments.

Furthermore, in the discussion we also mention that sediment heterogeneity at the shallow transect could also be derived from fishing events occurring in the area. Thus, although we did not measure directly bottom stress effects, we it find important to discuss the subject for the WIM, especially because of the amount of information already available for the area. In any way, we agree with the reviewer and we have rewritten the whole section.

Concerning the use of "high hydrodynamics" we have followed the suggestion of the reviewer. Thus, the term "high" was excluded from the text.

3) *"Finally the degree to which connectivity is analysed in the manuscript is pretty limited- connectivity between deep and shallow only measured apparently by cluster techniques."*

The reviewer is right. Considering that the success rate of our PCR amplification was low, we could not increase the amount of data used. However, we intended in this paper to corroborate with the results found by Bik et al (2010) conducted with the same genus and to give an insight that connectivity is possible for certain nematode genera in the deep sea. Of course, we tried to be careful with the interpretation of our results. In this paper, we could observe that there was no evidence for the lack of connectivity between the two transects, and not that connectivity is the rule for deep-sea nematodes.

Moreover, the use of cluster techniques was the only molecular method used for connectivity in this paper, together with the morphological analysis. For deep-sea nematodes, however, the use of other techniques, such as population genetics, is still in its infancy and even for 18S rDNA the results obtained cover a very small fragment of the DNA, and are mostly used to compare diversity between areas rather than more specific questions which require more refined techniques.

4) *“Furthermore, if I have followed the methods correctly, there are only 3 – 4 replicates from 6 and 4 stations (shallow and deep respectively) for nematodes (and it is unstated whether there is any replication for the other measures). I would have expected a study focussed on defining and explaining variability to have more replication and stations that that.”*

For this study we had six stations in the shallower transect with three replicates and four stations in the deeper transect with four replicates each, summing 34 cores which were studied. Other studies concerning spatial variability in the deep sea usually apply a similar number of replicates or less (for e.g. Danovaro et al., 2013 - Multiple spatial scale analyses provide new clues on patterns and drivers of deep-sea nematode diversity, Deep-sea research II, or Fonseca et al., 2009 - Regional patterns of nematode assemblages in the Arctic deep seas, Polar Biology). Moreover, the provided sampling is a maximum that could be achieved due to ship time of deep-sea samples. Considering that the community variation between cores from the same station was not significantly different, this means that each station in this study was robustly represented by the three-four replicates sampled. Finally, in order to support our choice of the amount of replicates, we have conducted a PERMDISP analysis, which resulted in a non-significant p-value, meaning that there was no dispersion of our data which could have influenced in the interpretation of our results due to replicate variability and dispersion effects.

For the environmental analyses we have included in the text that also 3-4 replicates were used according to the suggestion of the reviewer: “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.”

5) *I was surprised to see no reference to the Leduc et al paper “Nematode beta diversity on the continental slope of New Zealand: spatial patterns and environmental drivers."*

The reviewer is right. We have included the paper of Leduc et al. (2012) in the discussion, since it provides important information conducted at slopes as well.

6) *I would like to see a reduction in the scope of the manuscript with a focus on the variables actually measured (food availability) and the type of organism studied. Broadening the discussion to consider how different results may have been obtained if a greater mix of phyla, size and biological traits (including dispersal) had been studied would also be useful.*

We have followed the suggestion of the reviewer and decreased the importance of the hydrodynamics section in the paper. We have rewritten this section. Currently, the section about disturbance and hydrodynamics focused specially on the consequences of nematode resuspension and habitat colonization rather than hydrodynamics.

Considering the different phyla, we have discussed in (H4) how other groups, such as bivalves, gastropods, and crustaceans (amphipods and isopods) differentiate with increasing water depth along the slope in the following paragraph:
"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 genetic similarity across depth, suggesting taxon-specific barriers (Bik et al., 2010; Riehl and Kaiser, 2012). "

7) *I don't know that connectivity to the degree that it is analysed here is actually useful as there is no indication of space and time scales. The "connectivity" terminology should be dropped from the manuscript and instead merely discussed as a lack of clear depth-related population differences, depth-endemic lineages or isolation.*

We have followed the suggestion of the reviewer. The term connectivity was substituted in the discussion for the lack of isolation per habitat observed in this study, whenever it dealt with phylogenetic relationships. The (H4) of the discussion was also rewritten.

Yours faithfully,

Lidia Lins, on behalf of all authors

Evaluating ~~the importance~~environmental drivers of ~~Species variability and connectivity in the deep sea: evaluating effects of~~ spatial ~~heterogeneity~~ variability in ~~and for~~~~and hydrodynamics~~free-living nematode assemblage~~s~~s ~~in the deep sea~~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 ~~hydrodynamic effects and~~ surface primary ~~productivity~~productivity and ~~POC~~particulate organic matter flux to the seafloor, but also by heterogeneity in seafloor characteristics. ~~-~~However, to what extent these processes control ~~benthic~~ local ~~(α)~~ and regional ~~(β)~~ biodiversity ~~-~~remains~~s~~ unclear~~ due to the poor knowledge about species turnover in slope environment. s.~~ In this study, ~~we focused both local and regional benthic diversity was investigated on along (~30 km^2) area covering~~ two isobathic parallel transects located at the shelf break (300~~–~~–400 m) and upper slope (1000 m) of the Western Iberian margin ~~allowed~~ were used to test how ~~if~~ food ~~resources arriving at the seafloor~~ input and ~~habitat~~ sediment heterogeneity~~hydrodynamic effects~~ affect nematode ~~alpha and beta~~ diversity independently from spatial factors~~ at different depths in similar ways~~. We also examined the potential role of connec~~tivity~~tedness between both depth transects~~s by studying patch-dynamics of shared genera, and~~ through molecular phylogenetic analyses~~ as a result of dispersal~~. Local and regional ~~α d~~Ggeneric diversity and turnover were ~~treated as the diversity within a core, while β diversity was divided in~~ investigated at four levels: within a core, ~~beta diversity~~ within a station, between stations from the same depth transect, and between transects. H~~This is the first study applying integrative approaches using molecular and morphological techniques in combination with environmental factors to unravel spatial variability~~nematode distribution~~ and connectivity in relation to depth in the deep sea~~Western Iberian ~~. Results revealed that environmental variables were the main factor structuring benthic communities. h~~High variability in ~~resource ~~food availability and high sediment heterogeneity at the shelf-break transect were~~is~~ directly linked to high ~~α alpha~~and ~~β~~ diversity ~~between~~within stations, and~~ a~~ ~~ and spatial heterogeneity, promoting ~~higher variation in community structure across stations ~~(higher beta diversity) ~~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~~ β~~ between these deeper stations~~, which exhibited a rather low turnover due to the similar nematode community composition across upper-slope stations~~. Finally, ~~higher regional diversity was observed when comparing shelf-break and slope, indicating that ~~differences in nematode communities between both~~different~~ transects were more pronounced than differences within each of the isobathic transects, but these shifts were~~ in~~ paralleled by the~~ with outspoken~~ before-mentioned environmental shifts. These results suggest that changes in community structure are mainly dictated by environmental ~~differences ~~factors rather than spatial differences at the Western Iberian Margin. ~~suggestedand that communities dwelling in deeper regions 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~~Furthermore, phylogenetic relationships revealed no evidence for depth-endemic lineages~~ or isolation per habitat~~, indicating regular species interchanges across different depths.

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

1. Introduction

The link between biodiversity (i.e. species diversity~~ of species~~) and ecological processes (e.g. carbon flow, surface~~pecies~~ 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 (1000~~0~~–4000 m) encompass the largest habitat diversity in the marine environment (Levin and Dayton, 2009; Ramirez-Llodra et al., 2010). They harbour a high biodiversity, and are responsible for 90 % of the new biological productivity in oceans and seas, providing valuable food and energy resources for the marine fauna (Salgueiro et al., 2014).

It is generally accepted that ~~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 increasing commercial exploitation, ranging from fisheries, to gas, oil, and mineral extraction

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(Levin and Dayton, 2009; Puig et al., 2012). The direct impact of these unabated commercial activities on benthic environment and populations varies greatly, from pervasive sediment erosion, transportation and deposition, to the large-scale alteration of community composition (Puig et al., 2012). Therefore, continental margins comprise key locations to study the effects of environmental alterations on benthic biodiversity.

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

Food availability, as well as biological factors (predation, competition, dispersal), ~~are~~ assumed to drive small-scale (1–10 m²) patterns of benthic communities (Gage, 1997) promoting ~~alpha~~ local diversity (Levin et al., 2001). ~~Besides, other factors also play a role in shaping benthic communities.~~ ~~High c~~Community differences over large spatial scales (100–1000 m²; beta diversity) within continental margins ~~has~~ have been observed both along ~~a~~ bathymetric gradients as well as between different ~~stations~~ sites at ~~of~~ 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~~, 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, although ~~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). ~~dwelling~~ . ~~highly heterogeneous systems such as continental margins (Levin and Dayton, 2009).~~

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The lack of a pelagic larval stage in free-living nematodes, the focus group of this study, could therefore be viewed as a disadvantage to dispersal. Nevertheless, this abundant and omnipresent group of benthic metazoans is found at all depths and in all deep-sea habitats (Giere, 2009; Vincx et al., 1994). Nematodes belonging to the meiofauna (< 1 mm) exhibit high species richness and are one of the few taxa in which true cosmopolitan species may exist (Bik et al., 2010; Zeppilli et al., 2011). Some species are able to actively swim, following chemical cues, but more importantly, nematodes may be passively transported via water currents following resuspension from disturbance events (Jensen, 1981; Schratzberger et al., 2004). Molecular studies have indicated that different nematode taxa in diverse habitats exhibit population connectivity across a wide range, with some species showing subtle but significant genetic structuring at a small spatial scale, and other species exhibiting no differentiation along large distances (> 500 km). These findings confirm a high dispersal potential and low endemism for at least some species (Derycke et al., 2013, 2005). Nematodes therefore, hold ideal life traits when seeking to understand ~~connectivity~~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~~ This depth-range limitation ~~would contribute with~~ provides another explanation for why the bathyal holds such a high biodiversity (Rex and Etter, 2010). However, ~~W~~while empirical data for macrofaunal molluscs, crustaceans, as well as octocorals has been found ~~in~~ supporting ~~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).

Most previous research on nematode ~~alpha and beta~~ diversity has~~are~~ 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)~~(van gaever, zeppili)~~. No studies so far combined ~~a~~ bathymetric ~~and~~with geographic analys~~i~~es at a regional scale, which is crucial to understand patterns of biodiversity ~~at a regional scale~~. Furthermore, the transition from the shelf to the slope remains~~ed~~ a largely understudied area (Muthumbi et al., 2011; Vanreusel et al., 1992)~~(Vanreusel et al, 1992; Muthumbi et al., 2011)~~, while major environmental shifts are observed here. ~~and which may be realized by underestimated use of near-bottom currents as dispersal vectors~~

~~Using a small-scale design covering both transects located~~ Through the analysis of two isobathic transects of about 2~~2~~0 km length, ~~ong~~ and separated by 30 km and 600 m water depth, ~~in the same depth, as well as comparing different depth transects one can test~~ we tested ~~for both~~ the effect~~s~~ of environmental (food availability and sediment heterogeneity) and spatial ~~variables and spatial differences~~ ~~(e.g.~~ depth and geographical distance) variables on nematode ~~meiobenthic communities~~diversity. ~~. In this way, as environmental variables are expected to change with depth variation, more than with geographical changes, one would expect to see similar communities for similar depth transects but different communities across different depths. On the other hand, if spatial differences are more important and environmental variables are held constant for the same water depth, both differences within and between transects are expected at the same level, since stations vary spatially in both geographical and bathymetrical levels. .~~

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In this way, ~~study, this experimental design allowed for the first time to investigate how nematode community diversity changes was assessedat a small spatial scale (~30km) along two isobathic parallel transects located at different water depths (300 x 1000 m) at the Western Iberian Margin. Potential~~ drivers for turnover in nematode taxonomic composition were analysed at ~~three~~ four spatial scales: within a core, within stations, between stations from the same depth, and between ~~the~~ two depth transects~~s~~. 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 ~~potential~~possible depth-mediated differentiation, genus turnover and ~~connectivity~~ phylogenetic relationships ~~and~~through DNA sequence clustering between the two ~~bathymetric zones~~isobathic transects was investigated based on 18S rDNA sequence data of selected nematode taxa. The following hypotheses were tested: (H1) Higher~~The~~ patchiness of food resources deposited at the seafloor results in a higher local ~~alpha~~ diversity; (H2) ~~Disturbance (high hydrodynamics) i~~Increased~~s~~ ~~habitat~~ sediment heterogeneity ~~,~~ results~~ing~~ in a higher ~~beta diversity~~beta diversity; (H3) Beta diversity between different bathymetric transects is higher than beta diversity across similar depths; (H4) ~~There is connectivity~~Clades/Taxa are shared between ~~shallow~~ shelf break and ~~deep~~ slope areas.

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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 ~~hydrodynamics~~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).

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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~~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

Three to four replicated ~~S~~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., 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.

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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.

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2.3. Nematode sample processing for community analyses

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

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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):

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

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

2.4. Data analysis

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

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The nematode community data based on relative abundance of genera~~us level~~ 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)~~ and between all stations across both transects (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 et al., 2007). Subsequent pairwise pseudo *t*-tests were performed between all pairs of levels to determine where differences between each combination were found. Additionally, PERMDISP routines were used to test for homogeneity of multivariate dispersions between stations. PERMDISP results were not significant, indicating location differences through equally dispersed distances to centroids. SIMPER routines were executed based on Bray-Curtis similarity, with a cut-off of 90 % for low contributions. Dissimilarities within and between stations were compared with distances between geographical areas (km) and between depth differences (m).

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

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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.

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

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

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

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

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

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

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

3. Results

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3.1. Environmental parameters

Biogeochemical and granulometric properties of the sediment are shown in Figure 2. Nested PERMANOVA results for~~S~~ sediment particle-size diversity (SED) revealed ~~-~~significantly lower values ~~decreased~~ ($p < 0.05$) at the ‘deep’ transect. Pairwise comparisons for SED showed significant differences for the pairs [D2, D3] at the ‘deep’ transect and for all the pairs at the ‘shallow’ transect, except for [S1, S4], [S2, S614], and [S2, S613]. ~~The s~~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 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’~~‘deep’ stations were not significantly different from each other ($p > 0.05$) for % TOM, % TOC and % TN, while ‘shallow’~~‘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).

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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 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 (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], [S1, S614], [S7, S2], and [S2, S614]. Total nematode density was significantly higher at the 'shallow' stations when compared to the 'deep' stations ($p < 0.05$), but pairwise comparisons revealed no significant differences between pairs of stations (Fig. 4).

Dissimilarities between deployments within stations varied from 53–60 % (S1), 38–57 % (S2), 45–57 % (S4), 45–57 % (S613), 41–53 % (S614), and 42–58 % (S7) for the shallow stations, and 39–60 % (D1), 39–51 % (D2), 36–47 % (D3), and 44–64 % (D4) for the deep stations, thus revealing similar within-station variation for both transects. ~~In general~~Moreover, dissimilarity values between stations increased with increasing depth and geographical distance (Fig 5). SIMPER analysis revealed that the genera *Acantholaimus*, *Microlaimus*, *Richtersia*, and *Halalaimus* were mainly responsible for the average dissimilarity (63.99 %) between the two depths. The first genus was mainly found at 'deep' areas, whereas *Microlaimus* and *Richtersia* had higher densities at 'shallow' stations. The genus *Halalaimus* showed ~~constant average~~similar average densities in both transects, but higher density fluctuations at the 'shallow' stations. Nested PERMANOVA results showed significant differences between transects and among stations ($p < 0.05$) (Table S11). Pairwise comparisons revealed no significant differences between 'deep' stations, while the pairs of 'shallow' stations [S1, S7] and [S7, S2] possessed significant differences ($p < 0.05$) in nematode genera composition. ~~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~~ difference was observed for the shallow or deep stations ($p > 0.05$) (Fig. 4). Nested PERMANOVA results for relative abundance of trophic groups displayed significant differences between depths, but not among stations from the same depth ($p > 0.05$) (Table S12). Average similarity between 'deep' and 'shallow' areas was 80 %. SIMPER analyses revealed that differences between depths were mainly due to the higher relative abundance of selective deposit feeders (1A) at deeper stations. The shallow stations exhibited higher abundance of epistratum feeders (2A) and predators/scavengers (2B).

3.3. Correlation between nematode community structure and environmental variables

The correlation between univariate diversity values (H', J, EG (80), and TD) and environmental variables (% TN, % TOC, Chla, carotenes, CPE, sediment grain size, and SED) are shown in Table S13. Evenness (J') was not correlated to any

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environmental factor. Diversity (H’) was negatively correlated to silt-clay, but positively correlated to very fine-medium sand, SED, and total carbon and nitrogen. The EG (80) was negatively correlated to % TN, % TOC, and silt-clay, and positively correlated to CPE (Fig. 6), very fine sand, fine sand, medium sand, and SED (Fig. 6). Trophic diversity (TD) was negatively correlated to % TN and silt-clay, and positively correlated to Chla, CPE (Fig. 6), very fine sand, fine sand, and SED (Fig. 6).

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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 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 ~~generic~~ dissimilarity in genus composition between stations ~~in this study~~ along the Western Iberian Margin (WIM) were ~~are~~ higher at the ~~WIM~~ shelf break (‘shallow’ stations) when compared to the upper slope (‘deep’ stations). The upper slope nematode communities were in general dominated by the same genera (*Halalaimus* and *Acantholaimus*) ~~across stations~~, while at the shelf break not only ~~not only with high abundancesmore genera~~a higher diversity ~~were~~as present per station, ~~and~~but also the genus~~era~~ composition clearly differed between stations, resulting in a higher turnover. This higher station~~alpha~~ diversity and along transect~~beta~~ turnover at the shallower stations coincided with a higher amount and patchiness in food supply, as well as~~nd~~ a higher sediment heterogeneity within and between stations. However, the largest community differences and therefore highest taxonomic turnover in nematode genera was present between both depth transects~~sacross depth transectsNonetheless~~, despite ~~Nevertheless,~~ the high number of shared genera between transects and the intermingled pattern of genetic clustering observed for *Halalaimus* suggested ~~means~~ that ~~, while~~ depth ~~-~~as such did not restrict the distribution of ~~many taxa~~nematodes ~~as shown by the phylogenetic~~ ~~analysis~~.

~~4.~~

4.1. ~~(H1)~~ Sediment heterogeneity in combination with increased amount and ~~The~~ patchiness of food resources ~~deposited at the seafloor results in~~ contributes to a higher local ~~alpha~~ diversity ~~at the shelf break~~

In this study, local ~~-~~nematode ~~In this studyD~~diversity was significantly correlated with food availability ~~(Fig 6 give R).~~, ~~while also~~Moreover, ~~-~~the higher amount and variability of ‘labile’ food~~distribution~~ resources (Chla, CPE, and carotenes) within the ~~shallow~~ ‘shallow’ stations ~~(Fig. 2)~~when compared to the ‘deep’ stations (Fig. 2), ~~observed at the Western Iberian Margin (WIM)~~ was ~~positively~~ associated with a higher ~~alpha~~ diversity in terms of trophic group and genus composition ~~and genus community diversity~~ ~~(Fig. 4)~~. The interactions between coastline features and wind forcing already reported for the WIM, certainly affect the export flux, and consequently the resource distribution on the seabed. For this region, the strong surface dynamics may explain the high patchiness of food input and habitat~~d habitat~~ heterogeneity observed here, and consequently the high nematode local ~~alpha~~ diversity (alpha and beta within station) exhibited at the shallow stations (Cardinale et al., 2000; Crespo et al., 2011; Tokeshi, 1999). Concurrently, ~~The same A~~a different pattern~~however was not~~ was observed~~held~~ for the deeper transect~~, which exhibited much The interactions between coastline features and wind forcing already reported for the WIM, will affect the export flux and the distribution of labile organic matter on the seabed. Thus, the organic matter settling at the sea bottom mostly reflect the patchy patterns in surface primary productivity . In this regard, surface processes 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 variability of resources within and between deep stations, and~~, where the lower amount and variability of food resources were ~~and a lower variability as well as~~ associated with a decline in~~of~~ nematode ~~alpha~~ diversity ~~(Fig. 4)~~. A similar relationship

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between decreasing availability of ‘labile’ organic matter associated with~~to~~ a decrease in diversity was also observed ~~by Leduc et al. (2012b) for another slope~~ at similar depths in other slope environments (Danovaro et al., 2013; Leduc et al., 2012a; Netto et al., 2005) ~~at the New Zealand margin,~~ and may thus represent a general pattern at continental margins.

The generally low food input to deep-sea sediments~~in the deep sea~~ might favour organisms adapted to thrive in this food-deprived environment. In this study, ~~T~~the ~~potential~~ assumed deposit-feeding behaviour of *Halalaimus* ~~in this study~~ may explain its success in both transects and reflect its general high fitness in the deep sea ~~in general~~ (Fig. 3), because deposit-feeding can be interpreted as an adaptation to the ~~the~~ low availability and mainly refractory nature of ~~the~~ 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). ~~Despite exhibiting other feeding preferences, Tt~~The ~~presence~~ high abundances of the, as epistratum feeder classified, genus ~~*Acantholaimus*~~ *Acantholaimus* (based on buccal morphology by Wieser~~,~~ (1953)) ~~in higher abundances~~ at the deeper transect was also expected (Fig. 3), since *Acantholaimus* ~~this genus~~ is known as a ~~considered a~~ typical genus for the deep -sea (Miljutin and Miljutina, 2016; Muthumbi et al., 2011; Soetaert and Heip, 1995)~~a genus~~. It is indeed ~~already~~ widely observed that *Acantholaimus* ~~is~~ increases~~ing~~ in relative abundance from the shelf break towards abyssal plains, while it ~~and~~ dominates~~ing~~ nematode communities at all greater water ~~these~~ depths (Vanreusel et al., 2010). Until present,~~p to~~ 51 species of *Acantholaimus* were ~~already~~ described (Miljutin and Miljutina, 2016), ~~whil while while~~whileand~~and~~ a large number of different species ~~(up to 14 species)~~ have been found to~~can~~ coexist (~~-~~Muthumbi et al., 1997~~2011~~). By possessing a ~~large~~ great variation in its mouth parts, *Acantholaimus* species coexistence indicates a possible food resource partitioning ~~when food is not a limiting factor~~, 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 ~~along the upper slope can be~~ is possibly compensated by a higher species diversity and an associated flexible feeding strategy within the dominant taxa, such as *Acantholaimus*. ~~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.~~ The resource partitioning theory (Schoener, 1974) seems a plausible explanation for ~~genera~~the success of *Acantholaimus* with high inter~~ra~~-specific variation concerning feeding structures~~, which is not the case for most deep-sea nematodes~~.

~~either (cfr deposit feeders such as *Halalaimus*) oron a high degree of food partitioning (cfr specialised *Acantholaimus* species). In addition such as eses Fand flexible low ,ingnIn addition, changes in feeding habits according to the food input rate were also detected across different taxonomic groups of the deep-sea benthos (Amaro et al., 2009; Moens and Vincx, 1997) and for all benthic size classes (macro, mega-, and meiofauna) for which there is~~ .

~~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 et al., 2011). In addition, changes in feeding habits according to the food input rate were also detected across different taxonomic groups of the benthos (Amaro et al., 2009; Moens and Vincx, 1997).~~ However, t~~T~~he lower local diversity at the

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slope stations compared to the shelf break is not only explained by the lower and less patchy food input. ~~Similar to the patterns observed for food availability, s~~Sediment heterogeneity (SED) was also positively correlated with the expected number of genera and the trophic diversity at a local scale, exhibiting even stronger correlation results than the one between nematode diversity and CPE ~~, which was much less pronounced especially for the deep stations~~(Fig 6). The lower trophic diversity (TD) and higher relative abundances of deposit feeders in the deep stations already mentioned above, were potentially caused by a combination between low food input and comparatively higher sediment stability for this transect, reflected by the finer sediment composition in relation to the shallow stations (Fig. 2). This decline in TD with depth, positively associated with a decline in SED, was not observed in other studies along the slope, although the SED and TD values observed here were much higher than already reported before for other areas, and might reflect different~~ing~~ environmental conditions at the WIM (Danovaro et al., 2013; Leduc et al., 2012b; Pape et al., 2013).

The higher trophic diversity at the shallow stations suggests a higher niche differentiation within this transect, reflecting the possible transient stage of this habitat. Moreover, higher prominence of opportunistic species and fast colonizers ~~(epistratum feeders)~~, such as *Microlaimus*, was observed at the shallower WIM stations ~~in this study~~ (Fig. 3)~~), which were further characterized by higher food input~~.

The ~~dominance~~ 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), ~~where communities are dominated by few species during the~~ pointing to potentially ongoing ~~entire~~ recolonization processes following the disturbances~~s~~ (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~~s~~ regimes at the shallower stations and possible anthropogenic disturbance effects, for instance from~~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,~~So~~ sediment heterogeneity in combination with increased patchiness of food resources deposited at the seafloor observed here were associated with~~resulted in~~ a higher local generic and trophic diversity at the shelf break compared to deeper areas. Disturbance has been suggested before as a strong driver for diversity in the deep sea, with disturbances ranging from small-scale bioturbation traces, to intermediate-scale phytodetritus falls, or large scale currents~~.~~ (Levin and Dayton, 2009)~~(Levin and Dayton, 2009)~~. In our study, similar disturbances resulting in sediment heterogeneity and food patchiness at the shelf break, are used to explain the higher diversity compared to the 600 m deeper transect. Actually~~Indeed~~, the higher local diversity at the shelf break compared to the upper slope seems to contrast with the regular observed bathymetric diversity gradients for the deep sea, with mid-slope diversity maxima~~ as~~ recorded for multiple taxa~~.~~ (Levin and Dayton, 2009; Rex, 1981)~~(Levin and Dayton, 2009)~~. However, t~~Effects on the benthic fauna are mainly observed on deposit feeders (e.g. *Cervonema*, *Halalaimus*, *Daptonema*, and *Sabatieria*), with an increase in deposit feeding during transitional and depositional phases, when hydrodynamics or other disturbance effects decrease (Gage, 1997).~~

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~~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 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).~~

he higher diversity at genus level for nematodes- at the shelf break does not necessarily -imply ~~also~~ a higher species diversity for this environment, as~~. S~~ 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)~~(Muthumbi et al, De Mesel et al)~~. Still, generic diversity represents better the actual functional diversity for nematodes, ~~since~~as different genera are assumed to differ more in function than species within the same genus (Pape et al., 2013).

4.2. Greater ~~(H2) Disturbance (high~~ bottom ~~hydrodynamic~~stress~~) increases hS~~sediment ~~abitat h~~heterogeneity results ~~, resulting~~ in a higher beta diversity

In our study, ~~D~~distance-based linear models (DistLM) displayed a major significant effect of the silt-clay proportion on community variation when compared ~~amongst~~ between the two ~~distinct~~ 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~~ed~~ the generic diversity, and favours the dominance of the genera *Acantholaimus* and *Halalaimus*, commonly abundant in relatively undisturbed deep-sea soft sediments (Vanreusel et al., 2010). ~~Higher variation in sediment composition together with an increase in higher s~~Sediment partic~~e~~le-size diversity (SED) ~~at the 'shallow' stations were most probably possibly related to caused by strong near bottom current pulses already reported at the WIM (Quaresma et al., 2007)~~can be related to a ~~the~~ higher settling speed of coarser sediments, which can quickly settle out ~~in the deeper onshore~~ (Condie and Sherwood, 2006). Usually, the augmentation of bottom stress ~~can be~~ is responsible for eroding and suspending the local sediment, ~~which in turn separate~~ separating the dominant sandy fraction from the smaller silt-clay fraction~~s~~, which remains in suspension (Quaresma et al., 2007). Consequently, if higher resuspension rates of sediment can occur, ~~especially at depths shallower than 800 m~~ 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 ~~in relation to more stable environments (e.g. the 'deep' transect)~~. 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

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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).

~~Here, the increase in sediment and nematode community dissimilarity with increasing geographical distance and depth difference (Fig. 5) indicate a rapid taxonomic species turnover at the WIM.~~Ba the they inhabit~~(Condie and Sherwood, 2006)(Quaresma et al., 2007) Moreover, tThe higher dissimilarity for both nematode community and sediment diversity at the ‘shallow’‘shallow’ transect (Fig. 5), suggest higher bottom hydrodynamics in tthis areahis area that should be more likely to be subjected to rather strong near-bottom pulses that have been reported to regularly occur at the WIM (Quaresma et al., 2007) as well as fisheries, when compared to the ‘deep’ stations.. Near-bottom currents at the WIM, varying from 0.1–0.2 m s~~$^{-1}$~~, have the capacity to erode and suspend sediment (Condie and Sherwood, 2006; 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 in suspension (Quaresma et al., 2007)(Condie and Sherwood, 2006)Because (Condie and Sherwood, 2006; Tenzer and Gladkikh, 2014)under debate(Choe et al., 2012; Lins et al., 2013; Ullberg and Olafsson, 2003)have been shown to(Choe et al., 2012; Hockelmann et al., 2004) but may influence the location of settlement(da Fonseca-Genevois et al., 2006)~~

~~(Condie and Sherwood, 2006)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).~~

~~In addition~~Additionally to -the homogeneous silty sediments, also the persistent low food availability in the ‘deep’ transect ~~might~~reducess the environmental variability, and may consequently ~~may~~ limit the generic turnover. ~~To what extent similar patterns are present at species level remains uncertain. However, no trends were observed between beta diversity between stations and labile organic matter.~~ 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 ~~genus and~~ species level (Leduc et al., 2012b). ~~As already indicatedThus, although the deep sea exhibits,, the same trend and lack of correlation between beta diversity and food availability seems to hold (Miljutin and Miljutina, 2016; Muthumbi and Vincx, 1997)However t~~T~~T~~he 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 ~~that are~~ similar ~~in~~ gen~~era~~us composition can coexist in a food-depleted ~~, though which are each time typicaldeep-sea species richcommunities, can cotheadeep-sea~~ environment, whil~~e~~ere seemingly contrasting biodiversity at both local and regional scale ~~is enhanced with enhanced~~ increases with increasing food input~~, if no disturbance events occur~~. ~~Howeve~~In this sense~~r~~, when~~if~~ food input ~~is increased~~ ~~passes~~ reaches a~~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

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sea is in general higher (at the slope) compared to coastal sediments ~~(add refs)~~(Moens et al., 2014), despite the higher food input in shallow waters. ~~Still~~Nevertheless~~NeverthelessStill~~, in this study, ~~we confirm that the~~the higher sediment heterogeneity at the shelf break ~~is mainly responsible~~accounted the most for the ~~-~~higher generic turnover between stations from both slope and shelf-break transects.

~~Our study demonstrated higher trophic diversity (TD) and relative abundances of deposit feeders in the deep stations, potentially together caused bywith a comparatively higher hydrodynamic stability as indirectly inferred from sediment homogeneity, also and reflected also 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. (2012b) for similar depths, although the SED and TD values reported 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 several 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 assumed the relatively strong hydrodynamics regime and possible anthropogenic disturbance effects from fisheries at the WIM, which further 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 bemay result as a trade-off between the frequency of disturbance,~~

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~~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 ~~not~~ 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, However, the high silt-clay contribution together with the low variation within and between stations observed at the deep stations (Fig. 2) was associated with athe 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) and reduced the generic diversity.~~

In general, dissimilarity in genus composition increased with both depth difference and geographical distance. ~~(Fig. 5). Additionally in this investigation,~~ However, ~~station~~ dissimilarities ~~beta diversity~~ between depth transects ~~was higher toverlapped to a large extent in magnitude with the dissimilarities han within the shallow transects, although distances between stations and transects were comparable~~were larger than dissimilarities within transects, and genus turnover between~~at~~ the ‘shallow’ stations was greater than between~~turnover at~~ the ‘deep’ stations (Fig. 5~~1~~). If geographical distance would be an important factor, one would also expect a similar high turnover for the deep transect as observed for the shallow transect~~, implying that geographical distances were not so relevant in shaping nematode communities~~. Concerning water depth, ~~O~~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~~Here~~, the strong differences in sediment composition ~~and food resource patchiness~~ between transects (Figs. 5 and 7~~2~~) explained 33 % of the total nematode community variation between transects versus 3 % for water depth, appointing sediment differences as~~were~~ ~~likely~~ the main~~ factors~~ responsible factor for the higher~~er~~ beta diversity ~~observed~~ between transects ~~between the two transects~~rather than spatial differences. These findings suggest that diversity changes can be associated with both large and small-scale features driven by environmental alterations.

In general, beta diversity in the deep sea appears to be regulated by mechanisms of energy availability, biological interactions, disturbance, and habitat heterogeneity (Levin et al., 2001). Changes in these features at the slope occur much faster with increasing depth than with increasing isobathic distances (Rex, 1981), even for short distances, such as at the WIM study area~~. If distance was important one would expect also a high turnover for the deep transect~~. However here, still a considerate percentage of shared genera occurred between both transects and some genera exhibited similar abundances for both shelf break and upper slope, such as *Tricoma*, *Daptonema*, and *Halalaimus* (Fig. 3). In this respect, our results do not support the idea of isolation by depth between the shelf break and slope at the WIM area ~~(~~(Bik et al., 2010; Riehl and Kaiser,

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2012)~~add refs~~). ~~Also here~~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 ~~is~~are not ~~a~~the main cause for ~~differences~~variations in community composition.

~~Here, the increase in sediment and nematode community dissimilarity with increasing geographical distance and depth difference (Fig. 5) indicate the rapid taxonomic turnover from the shelf-break to the slope at the WIM is probably derived from the rapid settlement of organisms after being resuspended to the water column. Resuspension events and passive transport can occur not only alongshore but also along the slope, and this event might explain the shared nematode genera observed between the shelf-break and the slope.~~

~~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; Leduc et al., 2012a), 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 there are no major 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~~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 ~~be~~ indicat~~iv~~e ~~of connectivity between the two~~that bathymetrical isolation between the respective populations does not exist (Fig. 3). Yet, such depth-differentiation could occur

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on the intra-specific and species level. In our study, phylogenetic relationships within and between the genera sampled at both shallow and deep stations ~~potentially~~ 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, 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 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 ~~connectivity~~ genetic similarity across depth, suggesting taxon-specific barriers (Bik et al., 2010; Riehl and Kaiser, 2012).

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

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Even though our results indicate ~~connectivity~~ 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

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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 ~~much more~~ broader than here.

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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~~ 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.

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

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5. Conclusions

~~Our results reported a high variability of resource distribution (Chla, CPE, carotenes) and sediment composition within and between the shelf breakallow stations, associated with a high alpha and beta diversity at the WIM (Fig. 9). High local alpha diversity was mainly related to a complementary use of food resources (SED resulting in a high trophic diversity, whereereas h~~S~~igher hydrodynamics~~ ediment heterogeneity seemed to be the most important factor responsible for ~~at shallower habitats (inferred by the high sediment heterogeneity) promoted~~the greater variation in nematode community structure at all spatial levels studied: within stations, across stations, and between transects. ~~(higher beta diversity) compared to deeper areas,~~Deeper stations were uniformly dominated by silt-clay and exhibited low turnover and similar nematode communities typically found in deep-sea soft sediments, dominated by the genera *Acantholaimus* and *Halalaimus* (Fig. 9)~~ favouring organisms tolerant to disturbance to become more abundant~~. ~~Coarser~~ High sediment ~~composition~~ variability at the shallow stations was associated to a more diverse and contrasting nematode community between stations, where high turnover was characterized by the dominance of different genera at each station. This high sediment heterogeneity at the shelf break ~~indicated~~ suggests that strong near-bottom current pulses ~~can~~might be an indirect factor promoting diversity through the creation of patches and redistribution of resources, ~~have~~having thus positive effects on the benthic fauna~~, promoting diversity through the creation of suitable patches~~. Moreover, the high ~~The highher~~ beta diversity observed across transects ~~however does not~~

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surpassed the turnover at the shelf break and upper slope, and these differences were also 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 ~~highlights~~ of geographical distance and depth as regulating ~~spatial~~ factors~~ such as distance and depth~~~~the increase in stability and dominance of particular genera with increasing depth~~. 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.~~ To conclude,~~ Furthermore, phylogenetic ~~relationships~~ clusters suggested ~~connectivity between~~that ~~deep and shallow waters~~depth may not be a factor isolating populations of~~ for~~ the nematode genus *Halalaimus*.

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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

Figures

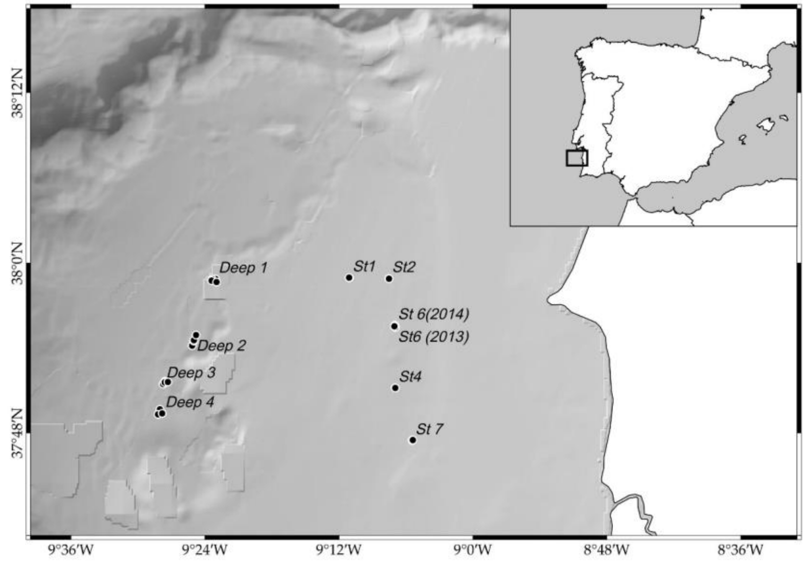
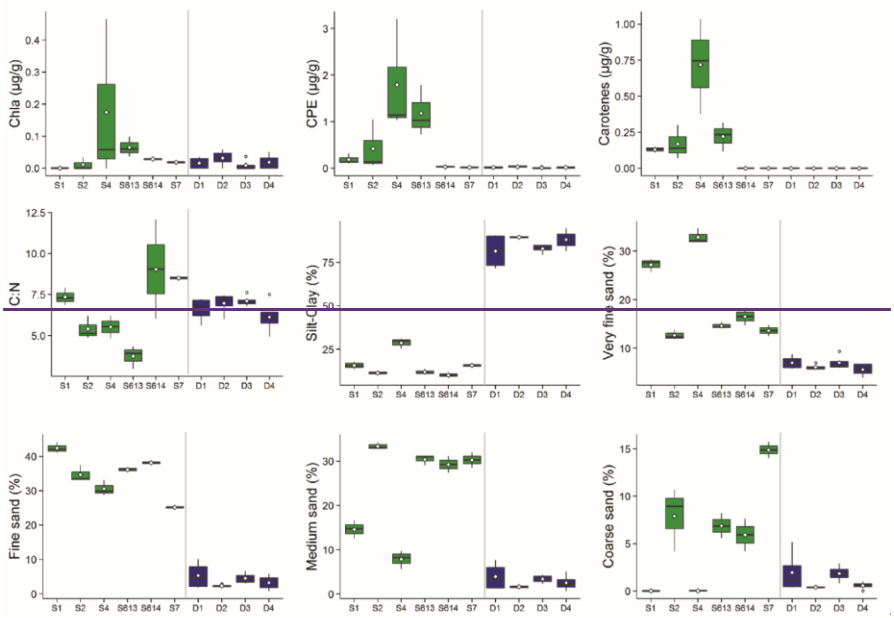


Figure 1. Location of B2013/17 and B2014/15 stations. Sampling stations are represented by black circles.

Field Code Changed

5


Chla (µg/g)
0.0
0.1
0.2
0.3
0.4
CPE (µg/g)
0
1
2
3
Carotenes (µg/g)
0.00
0.25
0.50
0.75
1.00
C:N
5.0
7.5
10.0
12.5
Silt-Clay (%)
25
50
75
Very fine sand (%)
10
20
30
Fine sand (%)
0
10
20
30
40
Medium sand (%)
0
10
20
30
Coarse sand (%)
0
5
10
15
S1 S2 S4 S613 S614 S7 D1 D2 D3 D4

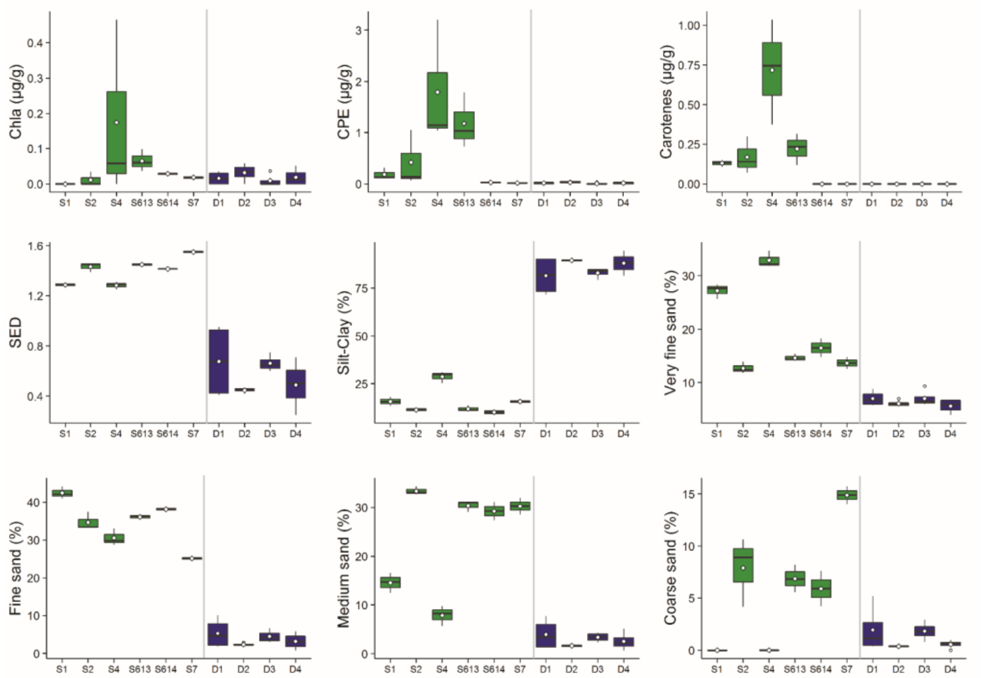


Figure 2. Environmental variables used in this study per station: Chl*a* (Chlorophyll a), CPE (Chloroplastic Pigment Equivalent), Carotenes, ~~C/N~~SED (~~ratio between % TOC/% TN~~sediment diversity), Silt-Clay, Very fine sand, Fine sand, Medium sand, and Coarse sand. Green boxplots represent shallow 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.

Field Code Changed

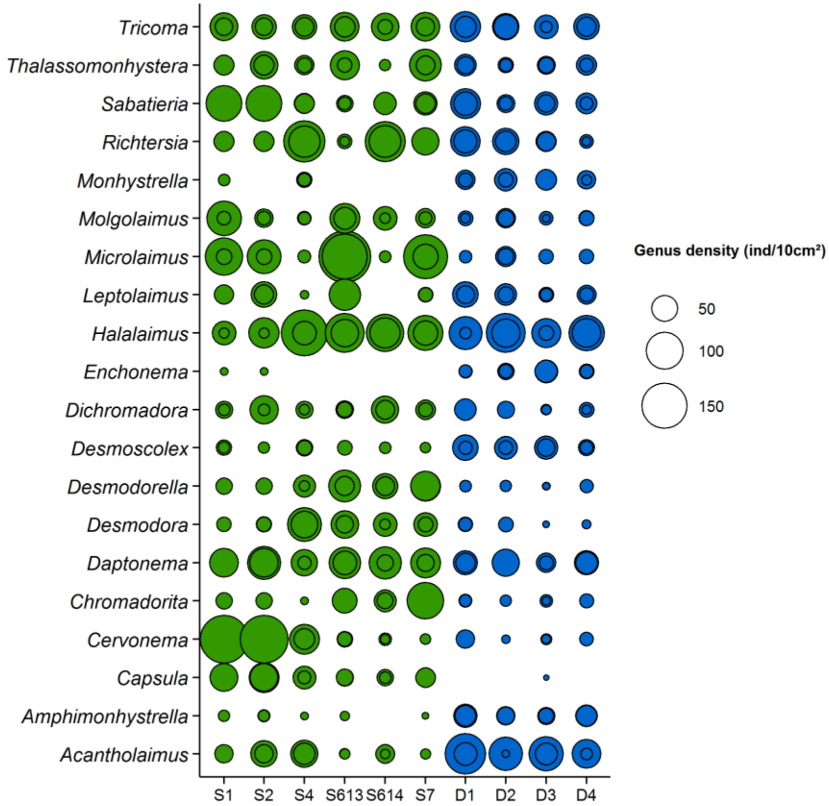


Figure 3. Most abundant nematode genera (> 4 %) per station. Inner circles indicate genus density (10 cm²) and outer circles genus standard deviation. Green circles represent shallow stations and blue circles deep stations.

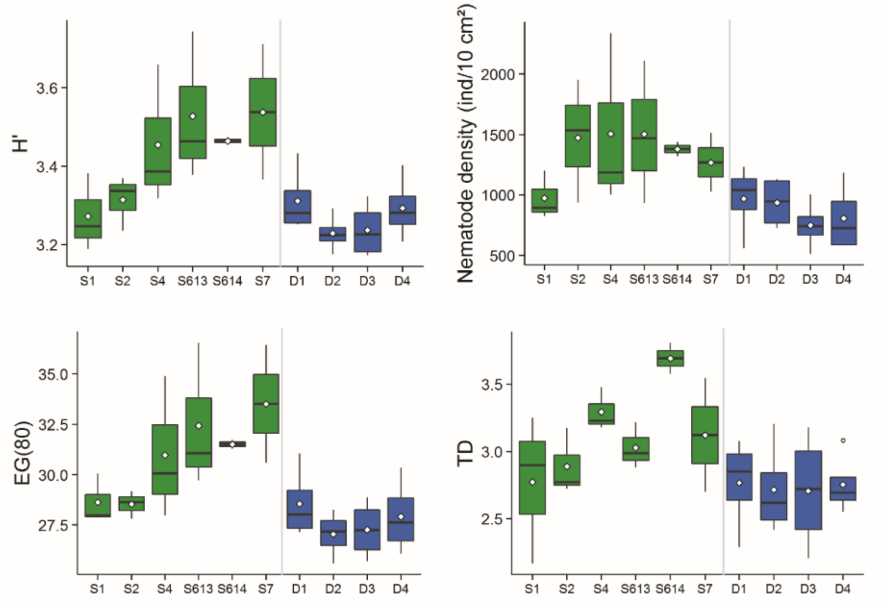
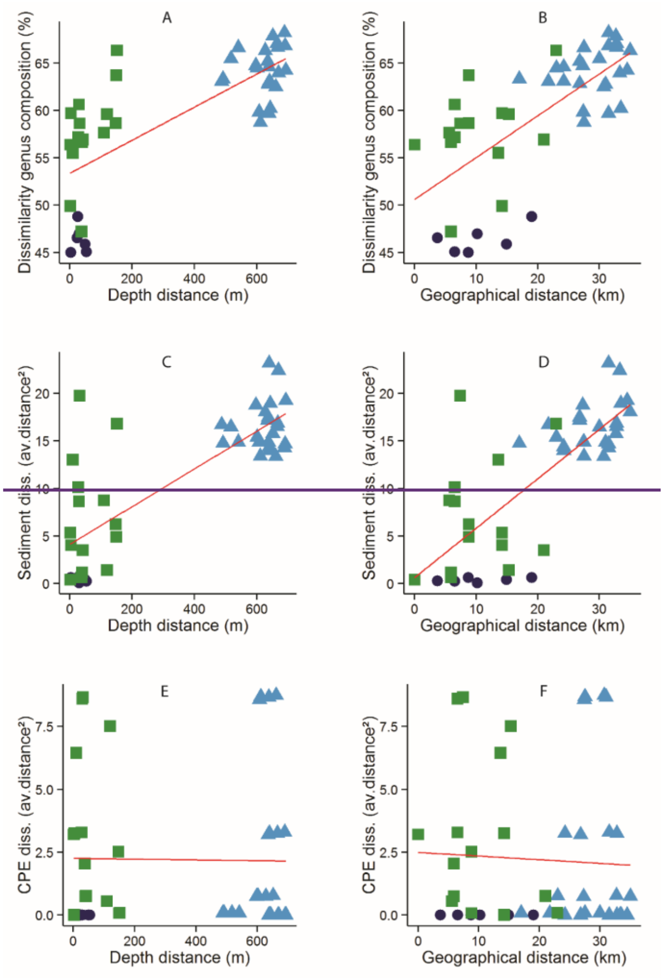


Figure 4. Nematode Shannon-Wiener (H') diversity, nematode density (individuals/10 cm²), Expected number of genera (EG(80)) and trophic diversity (TD) per station. Green boxplots represent shallow stations and blue boxplots represent deep stations. Black lines represent the median, empty circles represent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


A
Dissimilarity genus composition (%)
45
50
55
60
65
0
200
400
600
Depth distance (m)
B
Dissimilarity genus composition (%)
45
50
55
60
65
0
10
20
30
Geographical distance (km)
C
Sediment diss. (av.distance²)
0
5
10
15
20
0
200
400
600
Depth distance (m)
D
Sediment diss. (av.distance²)
0
5
10
15
20
0
10
20
30
Geographical distance (km)
E
CPE diss. (av.distance²)
0.0
2.5
5.0
7.5
0
200
400
600
Depth distance (m)
F
CPE diss. (av.distance²)
0.0
2.5
5.0
7.5
0
10
20
30
Geographical distance (km)

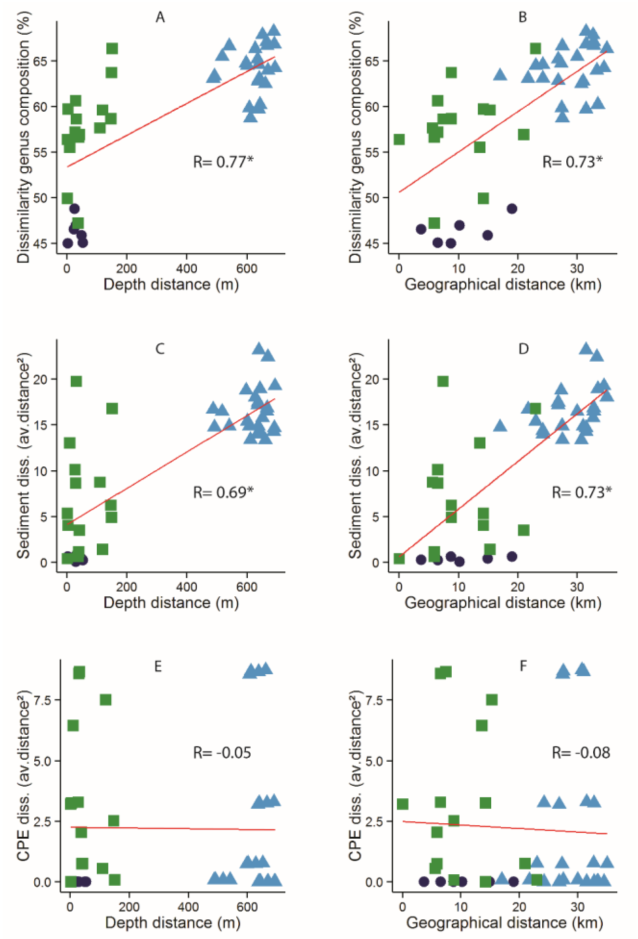
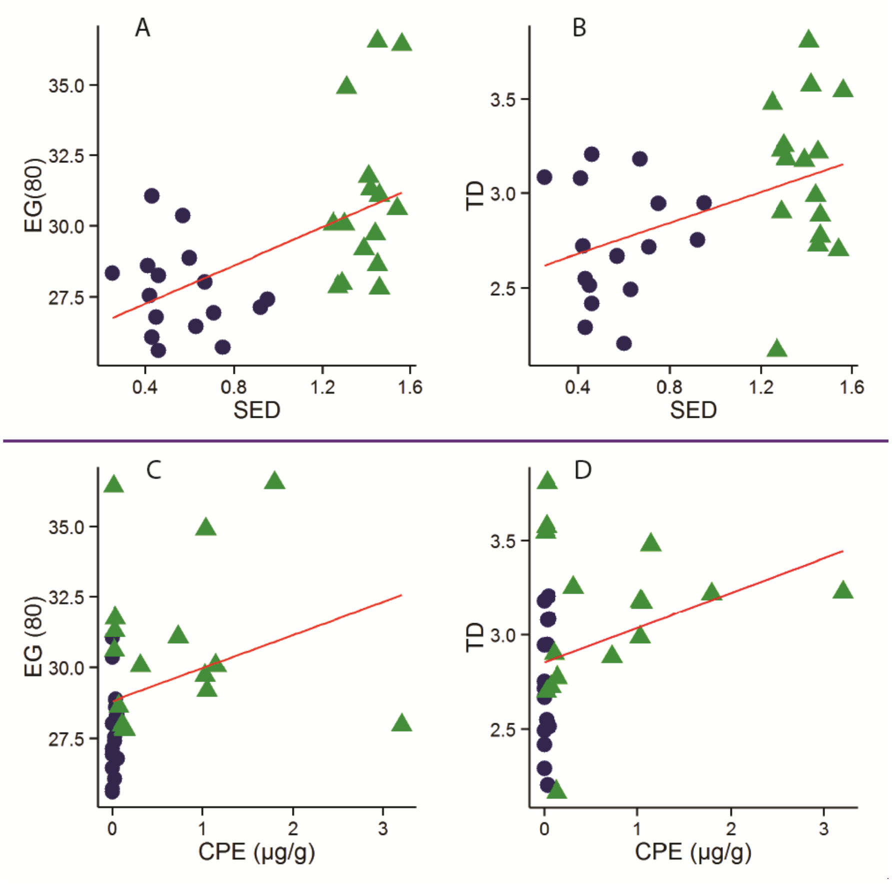


Figure 5. Dissimilarity values in nematode genus composition among depth difference (A) and between geographical areas (B), in sediment composition among depth difference (C) and between geographical areas (D), and in chloroplastic pigment equivalents (CPE) among depth difference (E) and geographical areas (F). Green squares represent dissimilarities between shallow stations, dark blue circles between deep stations and light blue triangles show dissimilarities between shallow and deep. Red lines indicate linear trends. . R indicate correlation values of Spearman. * shows significant correlations.


A
B
C
D
EG(80)
EG (80)
TD
35.0
32.5
30.0
27.5
3.5
3.0
2.5
0.4
0.8
1.2
1.6
SED
0
1
2
3
CPE (µg/g)

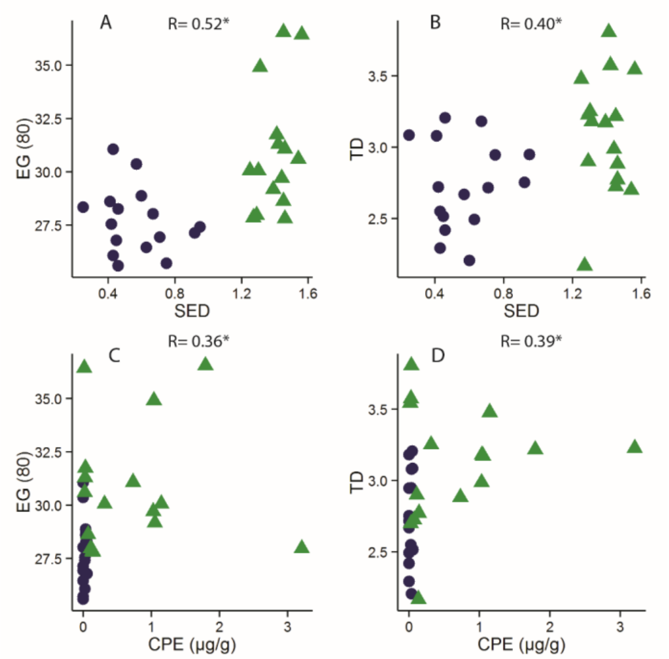


Figure 6. Correlations between (A) Sediment particle-size diversity (SED) and Expected genus diversity (EG (80)), (B) between SED and Trophic diversity (TD), (C) between EG (80) and Chloroplastic Pigment Equivalents (CPE) and (D) between TD and CPE. Green triangles represent correlations for ~~shallow~~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

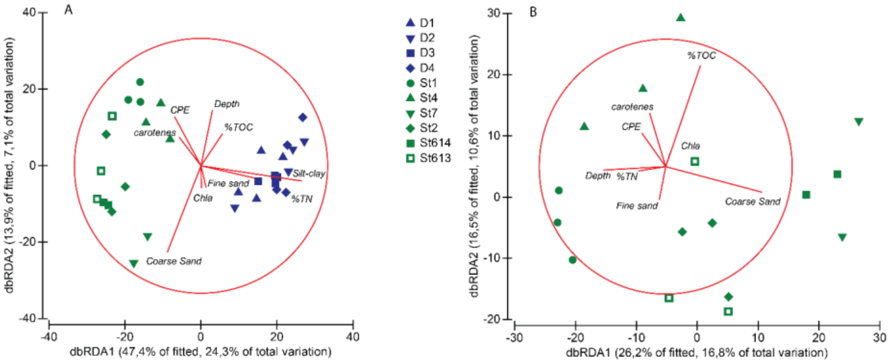


Figure 7. Distance-based redundancy analysis (dbRDA) illustrating the DistLM model based on the genera assemblage data for ~~“deep~~slope~~”~~ (blue) and ~~“shallow~~shelf-break~~”~~ (green) stations (A) and only between ~~shallow~~ shelf-break stations (B). Fitted environmental variables are shown with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

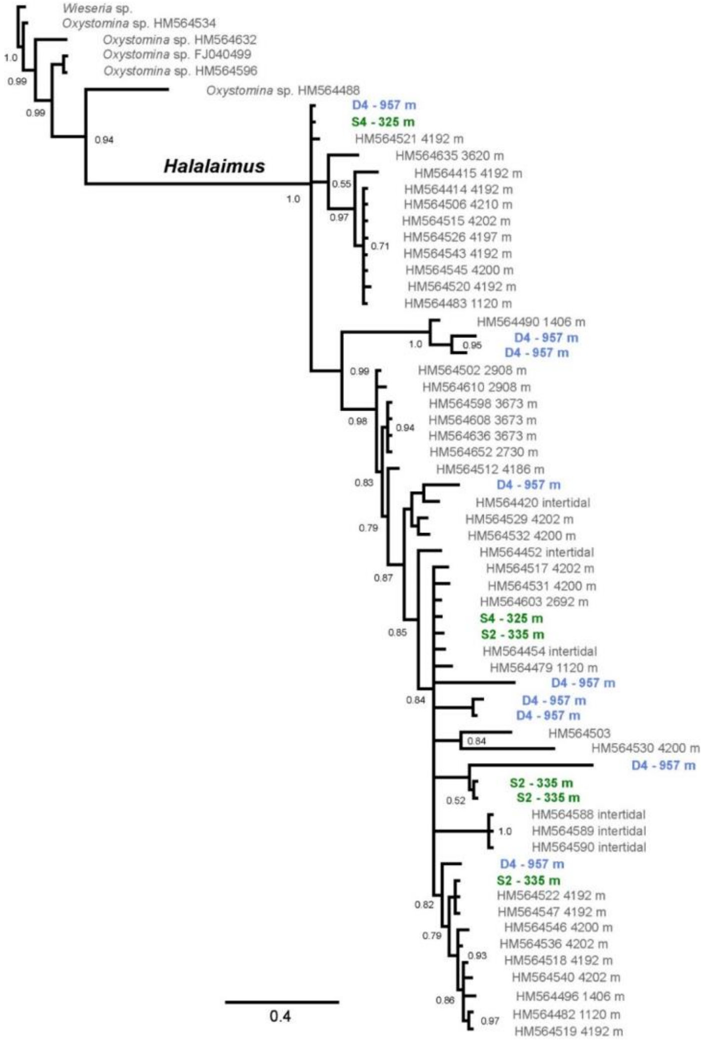
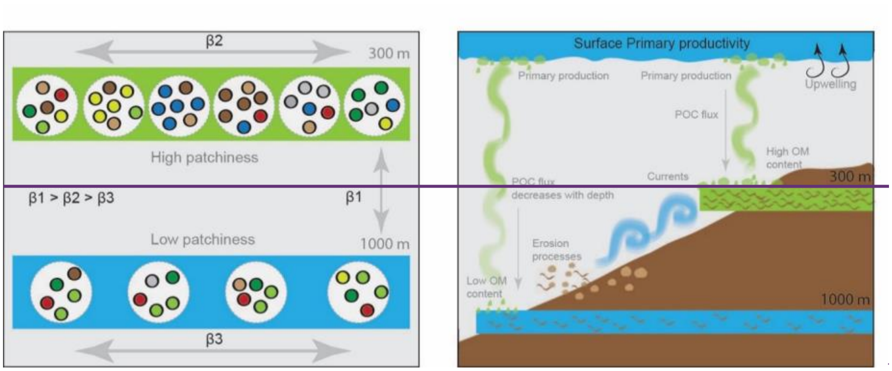


Figure 8. Consensus of Bayesian inference of phylogeny of the genus *Halalaimus* based on 18S rDNA sequence fragments generated in this study and from Bik et al. (2010); node support is given as posterior probabilities (PP); nodes with PP smaller than 0.50 were collapsed. The outgroup was set to *Wieseria*. The tree shows multiple instances of close relationships between individuals collected at different depth zones.



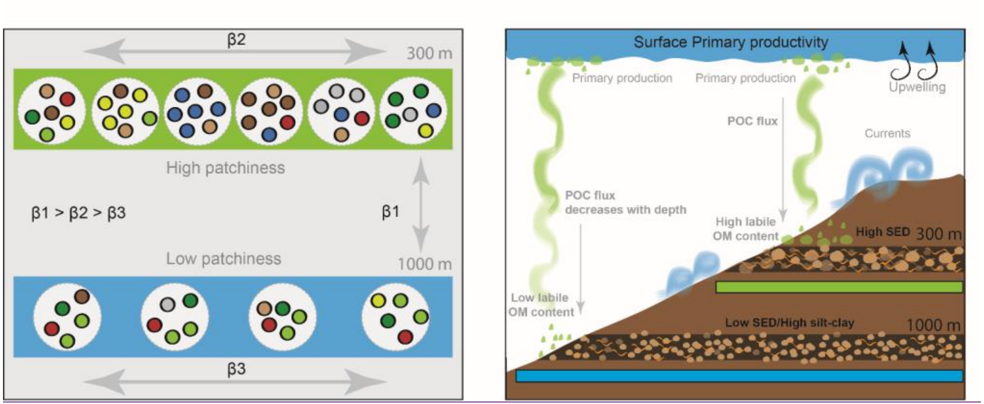


Figure 9. Scheme showing (left) how beta diversity varied across stations and between bathymetrical transects. The green bar represents the 'shallow' transect and the light blue bar the 'deep' transect.~~,~~ Grey circles inside the bars represents the stations sampled at each transect. Coloured circles inside grey circles refer to the variability in nematode genus composition within station and across stations. It illustrates ~~and~~ the higher densities and patchiness found at the 'shallow' stations and the lower turnover found at the 'deep' stations. ~~, and~~The (right) figure reveals 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~~local and regional diversity. The fate of organic matter produced at the surface varies with depth, where deeper areas will receive lower labile organic matter (OM) when compared to shallower areas. This figure also illustrates the higher variability in sediment at the 'shallow' transect when compared to the deeper transect. ~~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.~~The green bar represents the 'shallow' transect and the light blue bar the 'deep' transect.

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