Biodiversity and Function of Nematodes

in Contrasting Southern European Deep-sea Environments

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Biodiversity and Function of Nematodes

in Contrasting Southern European Deep-sea Environments

Biodiversiteit en Functie van Nematoden

in Contrasterende Zuid-Europese Diepzeegebieden

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Ellen

Maart 2013

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SUMMARY

Until around 150 years ago, the deep sea was regarded as a relatively uniform and stable habitat, and owing to the extreme abiotic conditions (lack of sunlight, high atmospheric pressure and low temperature), it was thought to contain no life. However, expeditions in the 1960s and 1970s revealed remarkably high biodiversity in the deep-sea realm; later, state-of-the-art technology (such as manned or autonomous submersibles) evidenced an unexpectedly high habitat heterogeneity and temporal variability. Although the deep sea (the pelagic and the benthic zone) constitutes the most extensive biome on Earth, it is still largely under-sampled and new habitats and species are still being discovered. There is a growing awareness of the global significance of this ecosystem since it hosts a large fraction of the Earth's biodiversity, contains a large reservoir of mineral and biological resources, regulates the climate (by taking up carbon dioxide, which is a greenhouse gas) and is an important player in the recycling of nutrients. Human activities (e.g. waste disposal, extraction of fish, minerals and hydrocarbons, global warming) have been shown to alter deep-sea ecosystem properties and processes. Hence, it is of the utmost importance that we advance our knowledge on the functioning of the deep-sea ecosystem to underpin its sustainable management. International, collaborative research projects like HERMES ("Hotspot Ecosystem Research on the Margins of European Seas", 2005-2009)/HERMIONE ("Hotspot Ecosystem Research and Man's Impact ON European Seas", 2009-2012) and BIOFUN ("BIOdiversity and ecosystem FUNctioning in Southern European Deep-sea environments: from viruses to megafauna", 2007-2011), which provided the frame for this doctoral research, were set up to gather more information on the structure, functioning and dynamics of deep-sea ecosystems.

Nematodes, which constitute the predominant meiofaunal phylum, are the most abundant, speciose and ubiquitous metazoan organisms residing in deep-sea sediments. Consequently, they represent the ideal organisms to evaluate macro-ecological patterns and to examine the link between diversity and ecosystem functioning. Because of their high standing stock, high metabolic and reproductive rates, and their presumed intermediate trophic position (between detritus and/or bacteria, and predatory meiofauna and/or macro- or megafauna) these roundworms are thought to have an important role in the benthic carbon cycle in coastal habitats. Whether this also applies to nematodes living in the deep sea is unknown, since information on their life history strategies in this extreme environment is completely lacking. So far, we have not succeeded in characterizing the feeding behaviour and preferences of nematodes, and also the functional importance of nematode activities that are unrelated to

foraging, remains a big unknown. An overview of the general characteristics of the deep-sea environment, the concept of biodiversity and the effect on ecosystem functioning, as well as the applicability to deep-sea nematodes is presented in **Chapter 1**.

The present PhD research intended to gain more insight into the drivers of nematode diversity at different spatial scales and in distinct environmental settings, and how this impacts their functioning within deep-sea ecosystems. This was achieved through field observational studies (**Chapters 2, 4 and 5**) and experiments (**Chapter 3**) which were carried out in several southern European deep-sea localities. The environmental drivers of nematode taxonomic and functional structure and diversity were investigated in both a cold seep system in the Gulf of Cádiz (Darwin mud volcano, **Chapter 2**) as well in deep-sea sediments showing no signs of chemosynthesis (in the Galicia Bank region and the Mediterranean, **Chapters 4 and 5**). At the Darwin mud volcano we evaluated small-scale (meters) heterogeneity in environmental conditions and nematode communities, whereas for the photosynthesis-driven habitats in the Galicia Bank region and the Mediterranean we studied regional (100s of kilometres) variation in local environmental and nematode community characteristics. Furthermore, we investigated the influence of the environmental regime and nematode community characteristics (structure and diversity) on ecosystem functions like the assimilation rate of dissolved organic matter and/or bacteria (**Chapter 3**) and total respiration rates (**Chapter 5**) of nematodes residing in photosynthesis-driven sediments.

In the first study (**Chapter 2**) we investigate the nematode community and trophic structure in relation to pore-water geochemistry at the Darwin mud volcano (Gulf of Cádiz, northeast Atlantic) along a 10 m transect from a seep site on the rim of the crater towards the mud volcano slope. Pore-water profiles indicated considerable variation in upward methane (CH_4) flow among sediment cores taken along the transect, with highest flux in the seep sediment core, gradually decreasing along the transect, to no CH_4 flux in the core taken at a 5 m distance. Low sulphate concentrations and high levels of total alkalinity and sulphide (H_2S) implied that anaerobic oxidation of methane (AOM) occurred close to the sediment surface in the seep sediment core. High H_2S levels had a genus and species-specific impact on nematode densities. Nematode genus composition varied gradually between sediment cores, with the genus *Sabatieria* dominating almost all sediment cores. Genus diversity increased with increasing distance from the seep site. These data suggest that the community structure of seep meiofauna is highly dependent on local (a)biotic characteristics, and a typical seep meiofaunal community cannot be delineated. Stable isotope values suggested the nematode diet up to 10 m from the seep site included thiotrophic carbon. The thicker hemipelagic sediment layer (a proxy for photosynthetic carbon input), the increased trophic diversity and the heavier nematode $\delta^{13}C$

values farther from the seep site point to a decrease in thiotrophy and an increase in photosynthetic carbon in the nematode diet.

Chapter 3 describes on-board experiments conducted with sediments from the Galicia Bank seamount and adjacent slope (northeast Atlantic), and from the western Mediterranean Sea. Sediment cores were injected onboard with ^{13}C-enriched dissolved organic matter (DOM) to evaluate nematode feeding ecology and the fate of DOM carbon in different benthic environments. We hypothesized that nematode ^{13}C label assimilation resulted from either direct DOM ingestion or preying on ^{13}C labeled bacteria. The time-series (1, 7 and 14 days) experiment conducted at the Galicia Bank seamount station was the first study to reveal a higher ^{13}C enrichment of nematodes than bacteria and sediments after a period of 7 days. Although isotope dynamics indicated that both DOM and bacteria were plausible candidate food sources, the contribution to secondary production and metabolic requirements of the nematodes (inferred from weight-dependent respiration rates) was higher for bacteria than for DOM at all stations. The seamount nematode community showed higher carbon assimilation rates than the northeast Atlantic and Mediterranean assemblages, which may reflect an adaptation to the food-poor environment. Our results suggested that the trophic importance of bacteria did not depend on the amount of labile sedimentary organic matter. Furthermore, there was a discrepancy between carbon assimilation rates observed in the experiments and the feeding type classification, based on buccal morphology. Stations with a similar feeding type composition (northeast Atlantic stations) showed large differences in uptake, whilst the nematode assemblages at the two slope stations, which had a differing trophic structure, took up similar amounts of the DOM associated carbon. Slope sediments were amended with glucose ("simple" DOM) or "complex" diatom-derived DOM to investigate the influence of DOM composition on carbon assimilation patterns. Our results did not evidence substantial differences in carbon processing related to the complexity of the DOM substrate. The quantity of processed carbon (5–42 % of the added amount of DOM) was determined by total consumer biomass (dominated by bacteria), and was primarily respired. The bulk of the added ^{13}C-DOM was not ingested by the benthic biota under study, and a substantial fraction was possibly adsorbed to the sediment grains.

The following two chapters deal with the variability in structural and functional characteristics of nematode communities alongside a longitudinal productivity gradient spanning the Galicia Bank region, the western and eastern Mediterranean basin.

In **Chapter 4** we show that the longitudinal reduction in surface primary productivity and in seafloor flux of particulate organic carbon (POC) is mirrored in the *in situ* organic matter

quantity and quality within the underlying deep-sea sediments at different water depths (1200, 1900 and 3000 m). Nematode standing stock (abundance and biomass) and genus and trophic composition were investigated to evaluate downward benthic-pelagic coupling. The longitudinal decline in seafloor POC flux was reflected by a reduction in benthic phytopigment concentrations and nematode standing stock. An exception was the station sampled at the Galicia Bank seamount, where despite the maximal POC flux estimate; we observed reduced pigment levels and nematode standing stock. The strong hydrodynamic forcing at this station was believed to be the main cause of the local decoupling between pelagic and benthic processes. Besides a longitudinal cline in nematode standing crop, we noticed a west-to-east gradient in nematode genus and feeding type composition (owing to an increasing importance of predatory/scavenging nematodes with longitude) governed by potential proxies of food availability (percentage of nitrogen, organic carbon, and total organic matter). Within-station variability in genus composition was elevated in sediments with lower phytopigment concentrations. Standing stock appeared to be regulated by sedimentation rates and benthic environmental variables, whereas genus composition covaried only with benthic environmental variables. The coupling between bathyal and abyssal nematode assemblages and surface water processes evidenced in the present study suggests that it is likely that climate change will affect the composition and function of deep-sea nematodes.

The bathymetric and longitudinal variation in several measures for nematode taxon (Shannon-Wiener genus diversity, expected genus richness and generic evenness) and functional diversity (trophic diversity, diversity of life history strategies, biomass diversity and phylogenetic diversity) is explored in **Chapter 5**. Our goals were to establish the form of the relation between diversity and productivity (measured as seafloor particulate organic carbon or POC flux), and to verify the positive and negative effect of sediment particle size diversity (SED) and the seasonality in POC flux (SVI), respectively, as observed for other oceanographic regions and taxa. In addition, we hypothesized that higher taxon diversity is associated with higher functional diversity, which in turn stimulates carbon mineralisation rates by nematode assemblages (determined from biomass-dependent respiration estimates). Taxon diversity related positively to seafloor POC flux. Phylogenetic diversity (measured as average taxonomic distinctness) was affected negatively by the magnitude and variability in POC flux, and positively by SED. The latter also related inversely to trophic diversity. Accounting for differences in total biomass between samples, we observed a positive linear relation between taxon diversity and carbon mineralisation in nematode communities. We could, however, not identify the potential mechanism through which taxon diversity may promote this ecosystem function, since none of the functional diversity indices related to both diversity and nematode respiration. These results suggest potential repercussions of climate change on deep-sea ecosystem functioning, but

further also emphasize the need for a better understanding of nematode functions and their response to evolutionary processes.

The final chapter (**Chapter 6**) integrates and discusses the main results from the preceding chapters. In addition, several suggestions for future, promising research avenues in deep-sea nematode ecology are presented.

Clearly, energy input, whether in the form of seafloor POC deposition (photosynthesis-driven habitats) or chemosynthetic bacteria (chemosynthesis-driven habitat), is an important structuring agent of deep-sea nematode communities. Unfortunately, the feeding experiments were unable to pinpoint either benthic bacteria or DOM as the primary food source for nematodes from the Galicia Bank region and the western Mediterranean. Besides food availability, chemical (high sulphide levels and anoxia) and physical (hydrodynamics) stressors, and habitat heterogeneity (grain size diversity) influence the structural characteristics of nematode assemblages. Importantly, the environmental context (energy input, chemical and physical stress, and habitat heterogeneity) does not only govern the structure, but also the function of nematode communities inhabiting deep-sea sediments. The observed impact of a climatically regulated upper-ocean process as primary productivity on the structure, diversity and function of deep-sea nematodes, implies that global warming is affecting the most prominent metazoan component of the deep-sea bottom.

SAMENVATTING

Meer dan 150 jaar geleden werd de diepzee beschouwd als een vrij stabiel en uniform habitat. Omwille van de extreme abiotische omstandigheden (gebrek aan zonlicht, hoge atmosferische druk en lage temperatuur) dacht men eveneens dat de diepzee geen leven herbergde. Echter, expedities in de jaren '60 en '70 onthulden een opmerkelijk hoge biodiversiteit in het domein van de diepzee. Verscheidene jaren later leverden hoog-technologische staalname-apparatuur (zoals bemande en onbemande onderzeeërs) bewijs van een ongekende habitatsheterogeniteit en temporele variabiliteit. Niettegenstaande de diepzee (de pelagische en benthische zone) de grootste bioom vormt op deze planeet, is deze enorm onderbemonsterd: nog steeds worden er nieuwe habitats en soorten ontdekt. Er is een toenemend besef van het globale belang van dit ecosysteem; de diepzee herbergt immers een groot deel van de biodiversiteit op deze aarde, bevat een grote voorraad aan mineralen en natuurlijke voedsel- en energiebronnen, regelt het klimaat en is een belangrijke speler in de regeneratie van nutriënten. Men heeft aangetoond dat antropogene activiteiten (zoals het dumpen van afval, exploitatie van vis, mineralen en koolwaterstoffen, en *global warming*) diepzee-eigenschappen en -processen veranderen. Daarom is het erg belangrijk dat we meer kennis vergaren over het functioneren van het diepzee-ecosysteem om zo een duurzaam beheer te onderbouwen. Internationale onderzoeksprojecten zoals HERMES ("Hotspot Ecosystem Research on the Margins of European Seas", 2005-2009)/HERMIONE ("Hotspot Ecosystem Research and Man's Impact ON European Seas", 2009-2012) en BIOFUN ("BIOdiversity and ecosystem FUNctioning in southern European deep-sea environments: from viruses to megafauna"; 2007-2011) werden op poten gezet om meer data te verzamelen over de structuur, het functioneren en de dynamiek van diepzee-ecosystemen. Deze projecten vormden het kader voor dit doctoraatsonderzoek.

Nematoden zijn het dominante fylum binnen de meiofauna. Deze wormen zijn de meest abundante, soortenrijkste en meest alomtegenwoordige meercellige organismen die leven in de sedimenten van de diepzee. Bijgevolg zijn zij de ideale onderzoeksorganismen voor het bestuderen van macro-ecologische patronen en het onderzoeken van de link tussen biodiversiteit en het functioneren van het ecosysteem. Omwille van hun hoge biomassa, hun intermediaire trofische positie (tussen detritus en/of bacteriën, en predatorische meiofauna en/of macro- en megafauna) en hun snelle metabolisme en reproductie worden nematoden geacht een belangrijke rol te vervullen in de benthische koolstofcyclus in kustsedimenten. Of dit ook geldt voor nematoden in de diepzee weten we echter (nog) niet, daar informatie over de levensgeschiedenisstrategieën in deze extreme omgeving ontbreekt. Tot nu toe zijn we er nog

niet in geslaagd hun voedingsgedrag en –vookeuren te karakteriseren. Ook het functioneel belang van nematodenactiviteiten die niet gerelateerd zijn aan het zoeken naar en nuttigen van voedsel blijft een groot mysterie. Een overzicht van de algemene eigenschappen van de diepzee, het concept van biodiversiteit en het effect op het functioneren van een ecosysteem, alsook de toepasbaarheid op diepzeenematoden wordt gegeven in **Hoofdstuk 1**.

Het doel van dit doctoraatsonderzoek was om meer inzicht te verwerven in de factoren die de diversiteit van diepzeenematoden sturen, en dit op verschillende ruimtelijke schalen en in afzonderlijke omgevingen. Daarenboven onderzochten we hoe hun diversiteit hun functie binnen het ecosysteem beïnvloedt. Deze doelstellingen werden bewerkstelligd met behulp van veldobservaties (**Hoofdstukken 2, 4 en 5**) en experimenten (**Hoofdstuk 3**) die uitgevoerd werden in verscheidene Zuid-Europese diepzeegebieden. De omgevingsfactoren die bepalend zijn voor de taxonomische en functionele structuur en diversiteit van nematoden werden onderzocht in een cold seep systeem in de Golf van Cádiz (de Darwin moddervulkaan, **Hoofdstuk 2**) en in diepzeesedimenten die geen tekenen vertonen van chemosynthese (in de regio van de Galicia Bank, in de noordoostelijke Atlantische Oceaan, en in de Middellandse Zee, **Hoofdstukken 4 en 5**). Op de Darwin moddervulkaan bestudeerden we de heterogeniteit in omgevingsvariabelen en nematodengemeenschapssamenstelling op kleine ruimtelijke schaal (meters). Voor de fotosynthese-gestuurde habitats in de Galicia Bank regio en de Middellandse Zee evalueerden we de regionale variatie (honderden kilometers) in lokale omgevingsfactoren en kenmerken van nematodenpopulaties. Bovendien gingen we na wat de invloed was van de omgevingsfactoren en gemeenschapssamenstelling en diversiteit van nematoden op ecosysteemfuncties zoals de assimilatie van opgelost organisch materiaal en/of bacteriën (**Hoofdstuk 3**) en totale respiratiesnelheden (**Hoofdstuk 5**) van nematoden die leven in fotosynthese-gestuurde sedimenten.

In de eerste studie (**Hoofdstuk 2**) onderzoeken we de gemeenschaps- en trofische structuur van nematoden in relatie tot de poriënwatergeochemie op de Darwin moddervulkaan (Golf van Cádiz, noordoostelijke Atlantische Oceaan) langsheen een 10 m lang transect tussen een seep site aan de rand van de krater en de vulkaanwand. Profielen van poriënwater duidden op een grote variatie in opwaartse methaan (CH_4) flux tussen de verschillende sedimentkernen verzameld langs het transect. De CH_4 flux was maximaal ter hoogte van de seep site, daalde geleidelijk aan langsheen het transect, en was onbestaande op 5 m afstand. Lage sulfaatconcentraties en hoge waarden voor alkaliniteit en sulfide (H_2S) impliceerden dat anaerobe oxidatie van methaan (AOM) plaatsvondt dicht tegen het sedimentoppervlak op de seep site. Hoge sulfideconcentraties hadden een genus- en soortspecifieke invloed op de nematodendensiteiten. De genussamenstelling varieerde lichtjes tussen de sedimentkernen,

waarbij het genus *Sabatieria* dominant was in bijna alle stalen. De genusdiversiteit steeg met toenemende afstand van de seep site. Deze dataset suggereert dat de gemeenschapsstructuur van seep meiofauna sterk afhangt van de lokale (a)biotische omstandigheden en dat een typische meiofaunagemeenschap niet gekarakteriseerd kan worden. Stabiele isotoopwaarden suggereerden dat het dieet van de nematoden thriotrofe koolstof bevatte tot op 10 m van de seep site. De dikkere hemipelagische laag (hetgeen een proxy is voor de input van fotosynthetische koolstof), de hogere trofische diversiteit en de zwaardere koolstofisotoopwaarden verder van de seep site wezen op een afname in thiotrofie en een toename in fotosynthetische koolstof in het dieet van de nematoden.

Hoofdstuk 3 beschrijft de experimenten die verricht werden met sedimenten van de Galicia Bank zeeberg en een naburige continentale helling (in de noordoostelijke Atlantische Oceaan) en van de westelijke Middellandse Zee. Aan boord van onderzoeksschepen werden sedimentkernen geïnjecteerd met ^{13}C verrijkt opgelost organisch materiaal (DOM) om de voedselecologie van nematoden en de bestemming van DOM in verscheidene benthische omgevingen te evalueren. We veronderstelden dat de opname van het ^{13}C-label door de nematoden het gevolg was van ofwel directe DOM inname ofwel van het eten van ^{13}C-gelabelde benthische bacteriën. Het tijdsreeksexperiment (1, 7 en 14 dagen) uitgevoerd op de Galicia Bank zeeberg was de eerste studie die een hogere ^{13}C aanrijking aantoonde in de nematoden dan in het sediment en de bacteriën over een tijdsspanne van 7 dagen. Niettegenstaande isotoopdynamiek zowel DOM als bacteriën als een plausibele voedselbron aanduidde, toch was de bijdrage tot de secundaire productie en metabolische behoeften (afgeleid op basis van gewichtsspecifieke respiratiesnelheden) van de nematoden steeds groter voor bacteriën dan voor DOM. De zeeberg-nematodengemeenschap vertoonde hogere koolstofassimilatiesnelheden dan de gemeenschappen van de continentale helling, hetgeen mogelijks een adaptatie is aan een lage voedselbeschikbaarheid. Tegen onze verwachtingen in leek het nutritioneel belang van bacteriën niet af te hangen van de hoeveelheid refractorisch organisch materiaal in het sediment. Daarenboven zagen we tegenstrijdigheden tussen de koolstofassimilatiesnelheden geobserveerd in de experimenten en de voedseltypeclassificatie gebaseerd op de buccale morfologie van de nematoden. Stations met een gelijkaardige voedseltypesamenstelling (de stations in de noordoostelijke Atlantische Oceaan) vertoonden grote verschillen in ^{13}C-assimilatie, terwijl de trofisch verschillende nematodenpopulaties van de continentale helling-stations een vergelijkbare opname kenden van het ^{13}C label geassocieerd met het DOM. Aan de sedimenten van de continentale helling werden zowel glucose ("simpel" DOM) als "complex" diatomeeën-afgeleid DOM toegevoegd om het effect van DOM samenstelling na te gaan op koolstofassimilatiepatronen. Onze resultaten toonden geen opmerkelijke verschillen in koolstofverwerking gerelateerd aan de complexiteit van het DOM. De hoeveelheid verwerkte

koolstof (5-24 % van het toegevoegde DOM) hing af van de totale biomassa (welke gedomineerd was door de bacteriën) en werd voornamelijk gerespireerd. Het overgrote deel van het toegevoegde DOM werd niet opgenomen door de bestudeerde benthische biota en een aanzienlijke fractie was waarschijnlijk geadsorbeerd door de sedimentpartikels.

De volgende twee hoofdstukken behandelen de variabiliteit in structurele en functionele karakteristieken van de nematodengemeenschappen langsheen een productiviteitsgradiënt tussen het Galicia Bank gebied, de westelijke en oostelijke Middellandse Zee.

In **Hoofdstuk 4** tonen we dat de longitudinale afname in primaire productiviteit en in de flux van particulair organische koolstof (POC) naar de zeebodem weerspiegeld is in de *in situ* kwaliteit en kwantiteit van het organische materiaal in de onderliggende diepzeesedimenten op verschillende waterdieptes (1200, 1900 en 3000 m). De biomassa, alsook de genus- en trofische samenstelling van nematodenpopulaties werden onderzocht teneinde de neerwaartse benthopelagische koppeling te evalueren. De longitudinale daling in de POC flux was gereflecteerd in een afname in benthische phytopigmentconcentraties en nematodenbiomassa. Een uitzondering op deze trend was het station op de Galicia Bank zeeberg, waar we gereduceerde pigmentconcentraties en nematodenbiomassa aantroffen ondanks de maximale schatting voor POC flux. Het heersende sterke hydrodynamische regime werd geacht de voornaamste reden te zijn voor deze lokale ontkoppeling tussen pelagische en benthische processen. Buiten een longitudinale vermindering in nematodenbiomassa observeerden we een west-oost gradiënt in de nematoden genus- en voedseltypesamenstelling bepaald door mogelijke proxies voor voedselbeschikbaarheid in het sediment (percentage van stikstof, organisch koolstof en totaal organisch materiaal). De variabiliteit in genussamenstelling binnen de stations was hoger bij lagere sedimentaire phytopigmentconcentraties. De biomassa van de nematoden leek voornamelijk gereguleerd te zijn door sedimentatiesnelheden en benthische omgevingsvariabelen, terwijl de genuscompositie enkel varieerde met deze laatste. De koppeling tussen diepzeenematodengemeenschappen en oppervlaktewaterprocessen die aangetoond werd in deze studie suggereert dat naar alle waarschijnlijkheid de klimaatsverandering de samenstelling en functie van diepzeenematoden zal veranderen.

De bathymetrische en longitudinale variatie in verschillende indices voor taxon (Shannon-Wiener genusdiversiteit, verwachte genusrijkdom en generische equitabiliteit) en functionele (trofische diversiteit, diversiteit van levensgeschiedenis-strategieën, biomassa-diversiteit en fylogenetische diversiteit) diversiteit van nematoden worden onderzocht in **Hoofdstuk 5**. Onze doelstellingen waren om de aard van de relatie tussen biodiversiteit en productiviteit (gemeten als POC flux naar de zeebodem) te achterhalen en om het positieve en negative effect van

respectievelijk, sedimentpartikeldiversiteit (SED) en de seizoenaliteit van de POC flux (SVI), zoals reeds waargenomen voor andere taxa en oceanografische regio's, te verifiëren. Bovendien veronderstelden we dat een hogere taxondiversiteit gepaard gaat met een hogere functionele diversiteit, waarbij de laatstgenoemde de koolstofremineralisatiesnelheden (bepaald aan de hand van gewichtsafhankelijke respiratieschattingen) van de nematodengemeenschappen stimuleert. Taxondiversiteit vertoonde enkel een verband met de POC flux naar de zeebodem. Fylogenetische diversiteit (gemeten aan de hand van de gemiddelde taxonomische divergentie) werd beïnvloed door de grootte van en de variabiliteit in POC flux, alsook door SED. De laatstgenoemde vertoonde eveneens een negatieve relatie met trofische diversiteit. Rekening houdende met verschillen in totale biomassa tussen de stalen, zagen we een positief lineair verband tussen taxondiversiteit en koolstofmineralisatie in nematodengemeenschappen. We konden echter niet het potentiële mechanisme identificeren waardoor taxondiversiteit deze ecosysteemfunctie zou promoten, aangezien geen enkele van de functionele diversiteitsindices gerelateerd was aan zowel taxondiversiteit als totale respiratiesnelheden. Deze bevindingen impliceren dat de klimaatsverandering mogelijks nadelige gevolgen heeft voor het functioneren van het diepzee-ecosysteem. Bovendien moeten we meer kennis vergaren inzake de functies van nematoden en hoe deze reageren op evolutionaire processen.

Het laatste hoofdstuk (**Hoofdstuk 6**) integreert en bediscussieert de belangrijkste resultaten van de voorgaande hoofdstukken. Bovendien worden er verschillende suggesties aangebracht voor toekomstige, veelbelovende onderzoekslijnen in het domein van de diepzeenematodenecologie.

Er is gebleken dat de input van energie, zowel in de vorm van POC flux (in de fotosynthese-gedreven habitats) als chemosynthetische bacteriën (in de chemosynthese-gedreven habitats) een prominente structurerende factor is van diepzeenematodengemeenschappen. Spijtig genoeg konden de voedingsexperimenten noch bacteriën noch DOM als de voornaamste voedselbron van nematoden in de Galicia Bank regio en de Middellandse Zee aanduiden. Behalve voedselbeschikbaarheid beïnvloeden chemische (hoge sulfideconcentraties en zuurstofgebrek) en fysische (sterk hydrodynamisch regime) stressveroorzakende factoren, en habitatheterogeniteit (korrelgroottediversiteit) de structurele eigenschappen van nematodenpopulaties. Een belangrijk punt is dat de omgevingscontext (input van energie, chemische en fysiche stress, en habitatheterogeneiteit) niet enkel de structuur maar ook de functie van nematoden in diepzeesedimenten lijkt te bepalen. De waargenomen impact van een klimaatsgereguleerd oppervlaktewaterproces zoals primaire productiviteit op de structuur, diversiteit en functie van diepzeenematoden suggereert dat de meest prominente meercellige

component van de zeebodem veranderingen ondergaat, en dit in de toekomst zal blijven doen, door toedoen van de klimaatopwarming.


Dave McCarthy

Chapter 1

General introduction

The general introduction provides background information on the setting (the deep sea), the specific research interests (diversity and ecosystem function and environmental drivers) and the organisms (nematodes) that are dealt with in this thesis. First, the general physical and biological features of the deep sea are mentioned, with an emphasis on the ecosystems considered in this work. Next, I highlight the different aspects of diversity, and clarify patterns documented for the deep sea. Then, the reader is informed on why and how biodiversity matters to the functioning of ecosystems and on the main insights gained from preceding marine and terrestrial biodiversity-function studies. Specifics on the diversity of deep-sea nematodes and their presumed function within the ecosystem are given. Subsequently, the collaborative research projects which provided the framework for this research and the study areas are introduced. At the end, the primary objective and the outline of the thesis are given.

1 The deep-sea environment

The deep-sea floor starts at the shelf break, which for most oceanic regions is located around 200 m water depth (Thistle 2003). Despite being the largest benthic biome on Earth (covering approximately 70 % of the Earth's surface), barely 5 % has been explored and the discovery of new habitats and species are ongoing (Ramirez-Llodra et al. 2010). The main reason for our inadequate knowledge of the deep-sea ecosystem is its remoteness requiring advanced marine technology and the financial resources for its development and usage.

Hitherto, scientific expeditions have revealed several general abiotic characteristics of the deep-sea benthic environment (listed by Gage and Tyler 1991, Thistle 2003). Large areas of the deep-sea floor are mainly covered with soft sediments. In areas close to land or at the base of canyons, sediments are partly derived from the weathering of continental rocks. In open oceans, sediments consist mainly of biogenic oozes originating from shelled planktonic organisms that live in the upper water layers. Rocky outcrops can be found in canyons, on seamounts or other slope areas where bottom currents or gas seepage blow away sedimented material (Gage and

Tyler 1991). Deep-sea water temperatures generally range between -1 °C and 4 °C, with the exception of bottom waters in the Red Sea (up to 21.5 °C) and the Mediterranean (13 °C), as well as near hydrothermal vents (up to 400 °C). Except for highly productive regions, oxygen concentrations in deep-sea waters are near saturation (5-6 ml l^{-1}). Salinity measures around 35 ‰, but can be over 39 ‰ in the Mediterranean and the Red Sea. Currents near the deep seabed are generally slow (< 10 cm s^{-1}); though in some regions velocities of up to 30 cm s^{-1} have been recorded. Another characteristic of the deep-sea environment is the absence of sunlight which can be used for photosynthesis, and as such the deep sea is an overall heterotrophic system.

2 Habitat heterogeneity in the deep sea

The deep seafloor was long considered to be a very uniform and relatively stable habitat. However, in the last few decades, advancements in marine technology (such as remotely operated vehicles or ROVs, submersibles, multibeam bathymetric mapping, coring devices) have stimulated deep-sea research, and (acoustic) imagery and video footage have shown this presumption to be far from true.

The continental margins signify the transition between the continents and the ocean (100 – 4000 m water depth) and represent around 15 % of the seabed (Fig. 1). The landward part of the margin, the continental shelf, stops at the shelf break, where a marked increase in the downward bottom gradient indicates the continental slope. On the ocean side, the continental slope is bordered by the continental rise, which is less steep compared to the slope. This physiographic zone connects the continental slope with the abyssal plain, which forms the largest part of the deep-sea bottom (estimated at 76 %; Ramirez-Llodra et al. 2010). Ecological depth zones used in deep-sea research are the bathyal (ca. 200 – 3000 m), abyssal (ca. 3000 – 6000 m) and hadal zones (> 6000 m). The bathymetrical limits of these zones vary slightly among studies; in this thesis, samples originating from 2400 m water depth or deeper were considered abyssal, while shallower sediments were considered bathyal.

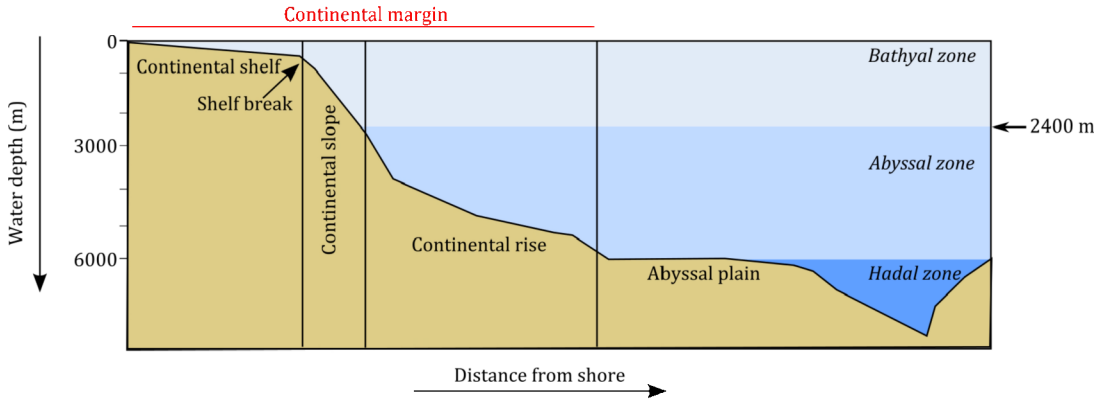

Fig. 1. *Cross-section of the ocean floor showing physiographic and ecological depth zones. Based on Thistle (2003).*

Continental margins constitute one of the most dynamic and heterogeneous environments on Earth. They comprise a diverse array of ecosystems generated by the interaction between water masses, tectonics, sediment diagenesis and terrestrial inputs (Fig. 2). Examples include cold seeps, carbonate mounds, canyons, oxygen minimum zones, cold-water coral reefs and seamounts (to name a few!). These habitats host different faunal communities, thereby contributing to the high biodiversity found on continental margins (Levin et al. 2010, Vanreusel et al. 2010b, Levin and Sibuet 2012). Besides the open continental slope, other margin habitats covered in this thesis are seamounts (which also occur on abyssal plains) and cold seeps, which are both elaborated on in the following paragraphs.

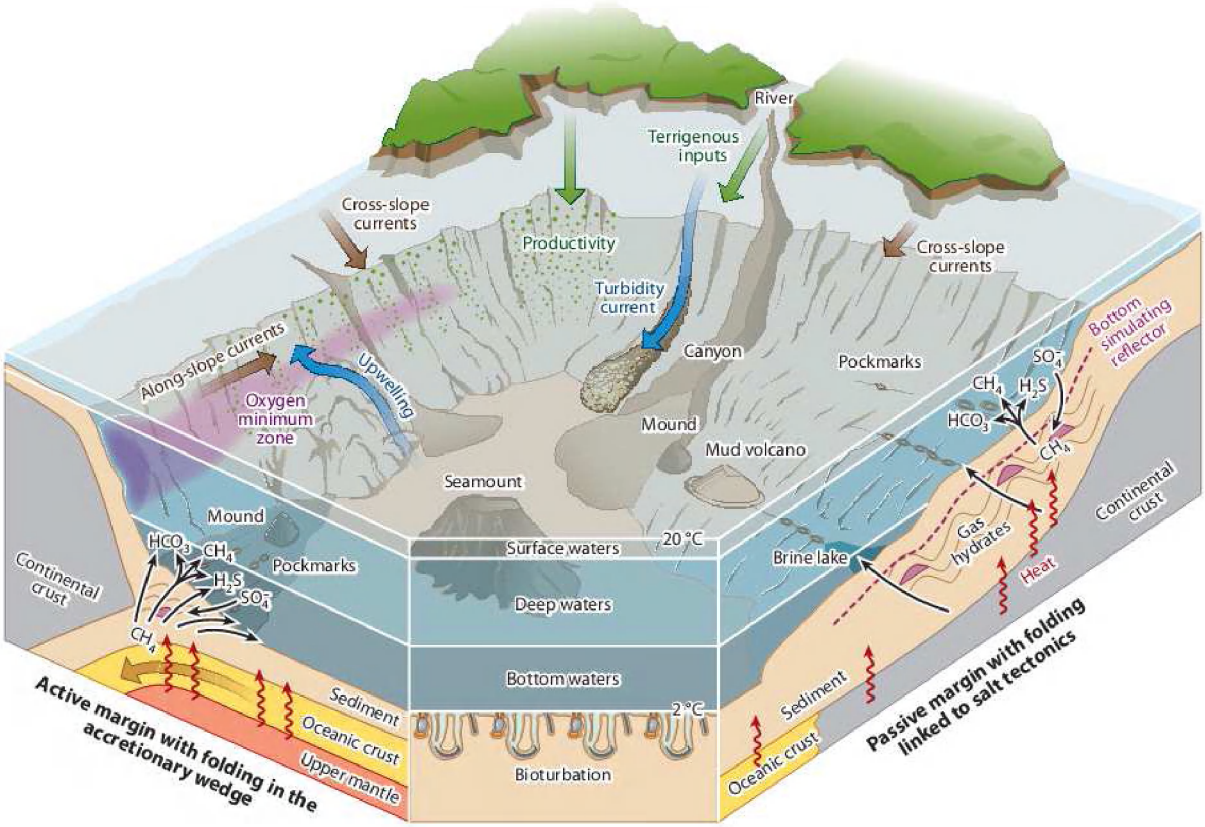

Fig. 2. *Scheme indicating the geological, chemical, and biological sources of habitat heterogeneity on continental margins. Not drawn to scale. From Levin and Sibuet (2012).*

Seamounts are undersea mountains, usually of volcanic origin, with a height of more than 100 m which do not break the sea surface (Rogers 1994, Consalvey et al. 2010). There may be as many as 3 million seamounts on the continental margins and abyssal plains, of which only a small percentage has been sampled for its biology (Consalvey et al. 2010). These topographic features represent an obstacle for ocean circulation, generating amplified tidal currents, jets and eddies, which influence the resident faunal community (Rogers 1994). Seamount studies have mostly addressed mega- and macrofauna, whereas comparatively little is known about meiofauna (Consalvey et al. 2010). There is a general belief that seamounts host distinct faunal communities with a high degree of endemicity (e.g. De Forges et al. 2000). However, the taxonomic composition of the fauna of most seamounts and the surrounding slope or abyssal plain is roughly similar and the higher number of endemic species occasionally found at seamounts may simply be the result of sampling bias (McClain 2007, Clark et al. 2010, Consalvey et al. 2010). Also the assertion that the water column atop of seamounts always experiences elevated primary productivity has been recently refuted (McClain 2007, Clark et al. 2010), and seamount secondary production seems to be supported by a mixture of autochthonous and

allochthonous energy sources (Rowden et al. 2010). Enhanced biomass of large pelagic vertebrates is regularly observed at seamounts, but there is little evidence for this pattern for benthic invertebrate organisms (Rowden et al. 2010). The field program "Census of Marine Life on Seamounts" (CenSeam, 2005-2010), which brought together scientists with differing expertise from all over the world, yielded much of the above insights into seamount ecology (Clark et al. 2012). However, given that seamounts are still largely under-sampled, much more research is required to fully unravel the structure and function of these ecosystems.

Although cold seeps occupy only a limited portion of the surface area of the world ocean (0.003 %; Ramirez-Llodra et al. 2010), they have received a relatively great deal of scientific attention owing to the extreme environmental conditions which often give rise to conspicuous megafaunal assemblages. These systems occur all around the world along active and passive margins (Sibuet and Olu 1998), and new locations are still being discovered (Levin 2005). Seepage can be associated with topographic depressions, i.e. pockmarks, but can also be found at topographic highs like mud volcanoes, mounds and mud diapirs[1]. Cold seeps are formed where methane gas or methane-laden pore water rises from seafloor sediments without a marked increase in the temperature of the overlying seawater. This methane can either have a thermogenic (transformation of deep buried organic matter under the influence of high temperatures) or a biogenic (microbial degradation of organic matter) source. The pore water of seep sediments can become enriched in H_2S through the anaerobic oxidation of methane (AOM) with sulphate serving as the final electron acceptor, according to the following net reaction:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$

This chemical reaction is carried out by a consortium of anaerobic methanotrophic (ANME) archaea and sulphate-reducing bacteria (Boetius et al. 2000, Knittel and Boetius 2009). AOM takes place at the sulphate-methane transition zone in anoxic sediments where the concave pore-water profiles of sulphate and methane intersect. The AOM zone is characterized by the production of H_2S and an increase in alkalinity, whereby the latter can lead to the precipitation of carbonates (Aloisi et al. 2002, Foucher et al. 2009, Knittel and Boetius 2009).

Owing to the spatial and temporal variability in seepage rates, geochemistry and substrate types, cold seeps constitute very heterogeneous environments. This heterogeneity in abiotic conditions gives rise to different microbial communities, symbiotic foundation species[2] and associated heterotrophic biota (Cordes et al. 2010). Examples of symbiont-bearing organisms typical of

[1] Positive seabed features composed of sediment raised by gas, which are smaller than mud volcanoes (Levin et al. 2005).

[2] Symbiont-bearing mega- and macrofaunal species which occur in seep environments and provide habitat for other fauna.

cold seeps include vesicomysid and bathymodiolin bivalves, and siboglinid tube worms (Sibuet and Olu 1998). At many cold seeps, faunal communities are characterized by an elevated standing stock, but lower biodiversity in comparison with adjacent non-seep sediments (Tunnicliffe et al. 2003, Levin 2005, Van Gaever et al. 2006, Vanreusel et al. 2010a).

Besides hosting a great portion of marine biodiversity, continental margins and abyssal plains perform important ecosystem functions such as carbon sequestration and nutrient cycling. Furthermore, these systems provide ecosystem services such as the production of food, fuel and minerals. Unfortunately, these systems are increasingly suffering from human activities like fishing, mining, waste disposal and last but not least, climate change (Thiel 2003, Ramirez-Llodra et al. 2010, 2011). Therefore, it is imperative that we investigate the drivers of biodiversity and the link between diversity and ecosystem functioning on continental margins and abyssal plains to provide guidelines which can support the sound management of this system (Levin and Dayton 2009).

3 Food in the deep sea: heterotrophic vs. chemosynthetic communities

Non-chemosynthetic deep-sea communities rely on organic matter produced in the euphotic zone that sinks through the water column and ends up at the seafloor. The processes that relate to the transport of organic matter produced in the sun-lit waters to the seafloor are collectively termed "the biological pump" (Ducklow et al. 2001). The particulate OM (POM) that is exported from the euphotic zone comprises phyto- and zoodetritus, in addition to bacteria, protists, faecal pellets (which mainly contain phytoplankton cells and gut bacteria) and inorganic compounds (Gage 2003). The efficiency of the biological pump (i.e. amount produced vs. amount transported) varies substantially between geographic regions (Lutz et al. 2002, Buesseler et al. 2007) and seasons (Lutz et al. 2007). The amount of POM that ultimately ends up at the deep-sea floor depends on water depth, and the sinking velocity (dependent on, amongst others, the degree of POM aggregation and the seawater mineral content) and decomposition rate (dependent on the pelagic food web structure and seawater temperature) of the POM particles (De La Rocha and Passow 2007). In addition, laterally advected water masses may transport sinking POM away from its point of origin (Gorsky et al. 2002, 2003, Zúñiga et al. 2007). The biological pump is inefficient since on average only 1 % of surface primary production reaches the deep-sea floor (Lutz et al. 2002), making this an energy-deprived environment.

The quantity and quality of settling organic matter is the most important determinant of the standing stock and composition of the deep-sea benthos (Ruhl and Smith 2004, Wei et al. 2010). The impact of pelagic processes, such as primary productivity and the resulting sedimentation, on the benthic ecosystem and vice versa is designated as benthic-pelagic coupling. Sedimentation events have been shown to affect the organisms associated with deep-sea sediments, manifested as enhanced biomass or metabolic activity (Danovaro et al. 1999a, Pfannkuche et al. 1999, Sommer and Pfannkuche 2000, Brown et al. 2001), or shifts in the vertical distribution within the sediment column (Shimanaga et al. 2000, Sommer and Pfannkuche 2000, Veit-Kohler et al. 2011). In addition to these observational studies, deep-sea organisms belonging to different phyla respond rapidly to an experimentally simulated phytodetritus pulse (Aberle and Witte 2003, Witte et al. 2003a, Moodley et al. 2005, Buhring et al. 2006, Jeffreys et al. 2010).

Along the vast energy-deprived deep-sea floor, there are relatively small, isolated oases where food is plentiful (Carney 1994). The faunal communities at these organic falls, hydrothermal vents and cold seeps rely on chemosynthesis, a process by which free-living or symbiotic prokaryotes oxidize reduced organic or inorganic compounds using chemical energy. The benthos living in these chemosynthetic habitats may either feed upon photosynthetically derived material deposited on the seabed (especially in shallow-water seeps) or upon local chemosynthetically fixed carbon. Stable carbon isotope signatures have been very useful in elucidating the dietary preferences of deep-sea organisms, including those inhabiting seep and vent habitats (Levin et al. 2000, Levin and Michener 2002, Van Dover et al. 2003, Van Gaever et al. 2006, Bergquist et al. 2007, Levin and Mendoza 2007, Thurber et al. 2010, Guilini et al. 2012). Very light carbon isotope signals ($\delta^{13}C \leq -50$ ‰) are indicative of a methanotrophic feeding mode, whereas consumption of sulphur-derived carbon yields heavier signatures ($\delta^{13}C$ values between -40 and -30 ‰ or between -9 and -16 ‰, depending on the type of Rubisco enzyme that is used to fix CO_2 in the Calvin cycle) (Robinson and Cavanaugh 1995). More recently, the fatty acid composition, whether or not combined with stable carbon isotope analysis, has also been examined to reconstruct the diet of organisms living in chemosynthetic ecosystems (MacAvoy et al. 2002, Colaço et al. 2007, and Van Gaever et al. 2009b). At the Håkon Mosby mud volcano (Arctic Ocean), nematodes were found to feed primarily on free-living sulphur-oxidizing bacteria (Van Gaever et al. 2009b), while at cold seeps at the Blake ridge in the south Atlantic (Van Dover et al. 2003) and at the Cascadian margin in the northeast Pacific (Guilini et al. 2012), they potentially consumed methane-derived carbon. Remarkably, even when a seep or vent community is observed to feed exclusively on chemosynthetic carbon, these communities are not entirely independent of photosynthesis. Both dissolved oxygen, which is required for the

metabolism of all metazoans and many prokaryotes, and methane fuelling cold seeps have their origin in photosynthesis (Tunnicliffe et al. 2003).

4 Biodiversity

Biological diversity or biodiversity can be characterized at various organizational levels, namely genotypes, taxa, functional groups, and landscapes. Generally, biodiversity is determined as the number of taxa (i.e. taxon richness), mostly assessed at the species level, that are living in a given areal unit. As a consequence of limited sampling effort, the total number of species inhabiting this planet and its oceans is a big unknown. Recent estimates of the total number of eukaryotic marine species range between 0.5 (Appeltans et al. 2012) and 2.2 million (Mora et al. 2011). In what follows, we will use the term taxon diversity to indicate both species and genus diversity.

4.1 Assessment of taxon diversity

Taxon diversity covers three aspects: the number of taxa present (i.e. richness), the distribution of individuals over these taxa (i.e. evenness), and the degree of relatedness amongst taxa (i.e. taxonomic divergence or distinctness). There are many biodiversity indices, each of which highlights different aspects of diversity (e.g. Hurlbert 1971, Hill 1973; reviewed by Heip et al. 1998, Maurer and McGill 2011), and so it is advisable to calculate a suite of indices to characterize taxon diversity within a given locale (Levin et al. 2001). In the study of (deep-sea) nematodes, the most commonly applied diversity measures are Hill's diversity numbers of differing order (N_a with a indicating the order, which ranges between zero and infinity), (expected) taxon richness, Pielou's evenness, and Shannon-Wiener diversity. Many diversity indices depend upon the number of individuals considered; only expected taxon richness, average taxonomic distinctness, variation in taxonomic distinctness and, to a lesser degree, N_{inf} are sample-size independent (Soetaert and Heip 1990, Clarke and Warwick 1998, Clarke and Warwick 2001b). Diversity can be described on varying spatial scales from a single point (i.e. one sample) to biogeographical provinces (Gray 2000). The present work deals with single point diversity measures only.

4.2 Deep-sea taxon diversity

Owing to the limited food availability, the high water pressure and low temperature, the deep sea was long thought to be very species poor, or even completely devoid of life. Only a few decades ago, improved sampling has revealed unexpectedly high species diversity in the deep sea (Sanders and Hessler 1969, Grassle 1989, Grassle and Maciolek 1992). This high level of local diversity is established and supported by historical evolutionary and contemporary ecological processes operating across different spatial scales (Levin et al. 2001, Rex and Etter 2010).

Local diversity within deep-sea sediments is not spatially uniform but varies amongst and within oceanographic basins. There are two assumed geographic gradients in benthic biodiversity. The first, which is not explored further in this work, is the latitudinal diversity gradient. Several deep-sea taxa become more speciose with decreasing latitude; evidence for this pattern in the southern hemisphere is less convincing (Culver and Buzas 2000, Brandt et al. 2007). Both evolutionary and ecological processes (mainly nutrient input) have been proposed as potential causes of the latitudinal diversity cline (Stuart et al. 2003). In addition to a monotonic latitudinal gradient, marine biodiversity often displays a unimodal bathymetric trend with a peak at the mid- to lower continental slope (reviewed by Rex and Etter 2010). However, this unimodal pattern is not observed is not observed for all taxa or regions (see examples in Stuart et al. 2003, Rex and Etter 2010). The variability in benthic diversity with water depth is not the pure consequence of taxon boundary constraints imposed by the coastal and the abyssal environment (Levin et al. 2001, Rex and Etter 2010). Multiple simultaneously acting ecological processes, such as productivity and the mediation of biological interactions, physical disturbance, and sediment heterogeneity have been invoked as possible drivers of the diversity-depth curve (Stuart et al. 2003, Rex and Etter 2010).

This doctoral thesis investigated whether nutrient input (inferred from modelled seafloor particulate organic carbon flux) and/or sediment heterogeneity govern the bathymetric and longitudinal variability in nematode diversity along a transect spanning the Galicia Bank region and the Mediterranean basin. This west-to-east transect was believed to represent a negative gradient in the amount of food available to the benthos, based on (1) the decline in primary productivity between the region of the Galicia Bank and the Mediterranean (compare Joint et al. 2002 with Bosc et al. 2004), and between the western and eastern Mediterranean basin (Turley et al. 2000, Siokou-Frangou et al. 2010), and (2) the higher water temperatures in the Mediterranean facilitating the breakdown of sinking organic material. Since the flux of photosynthesis-derived organic matter decreases with depth (Lutz et al. 2007), also water depth can be regarded as a proxy for food availability at the seabed. At lower productivity levels (i.e.

the ascending part of the unimodal diversity-productivity curve), nutrient input is thought to promote diversity through the stimulation of population growth rates of individual species. The depressed diversity under high productivity regimes (the descending limb of the curve) may have several reasons; a higher rate of competitive exclusion is the most often invoked explanation (Rex and Etter 2010). Besides a decline in nutrient input, bathymetric diversity trends may reflect gradients in sediment particle size diversity, and so we also evaluated the impact of sediment heterogeneity on nematode diversity. The previously reported positive relationship between macrofaunal (Etter and Grassle 1992) and nematode (Leduc et al. 2011) species diversity and sediment heterogeneity is presumed to be driven by higher small-scale resource variability or habitat complexity.

4.3 Functional diversity

There is a growing amount of research that considers the diversity of organism traits or functional diversity (Petchey and Gaston 2002, Danovaro et al. 2008a, Cadotte et al. 2009), instead of, or complementary to pure taxon diversity, because it provides a direct mechanistic link between the diversity of taxa and ecosystem functioning (Walker 1992, Bengtsson 1998, Díaz and Cabido 2001). In other words, there is no direct relation between the variety of taxa and the functioning of ecosystems, and it is the functions performed by these taxa (related to the type and range of functional traits) that determine ecosystem properties and processes (Fig. 3). Several studies have revealed a positive effect of functional diversity on ecosystem functioning (Danovaro et al. 2008a, Cadotte et al. 2008, 2009, Flynn et al. 2011), although some found no discernible impact (Norling et al. 2007, Flynn et al. 2011). Two hypotheses have been put forward to explain the positive influence of functional diversity on ecosystem properties and processes; i.e. the complementarity hypothesis, which states that a higher functional diversity should lead to a higher complementarity in resource use, and the insurance hypothesis, according to which higher trait diversity implicates a higher resilience against environmental perturbations. Correspondingly, functional traits can be subdivided into effect functional traits (which determine the impact on ecosystem processes, e.g. feeding mode in infaunal organisms, which may influence nutrient fluxes) and response functional traits (which govern the response to environmental change, e.g. drought tolerance in plants) (Díaz and Cabido 2001, Hooper et al. 2002, Naeem and Wright 2003). Some functional traits belong to both classes; for instance, the size of infaunal organisms determines locomotion mode (which may impact nutrient fluxes and thus has a functional effect) and vulnerability to predation (a wider variety in predator vulnerability may govern higher temporal stability, and thus determines the functional response). Most marine and terrestrial biodiversity-function research has focused on effect

functional traits (Hooper and Vitousek 1997, Emmerson et al. 2001, Norling et al. 2007, Danovaro et al. 2008a).

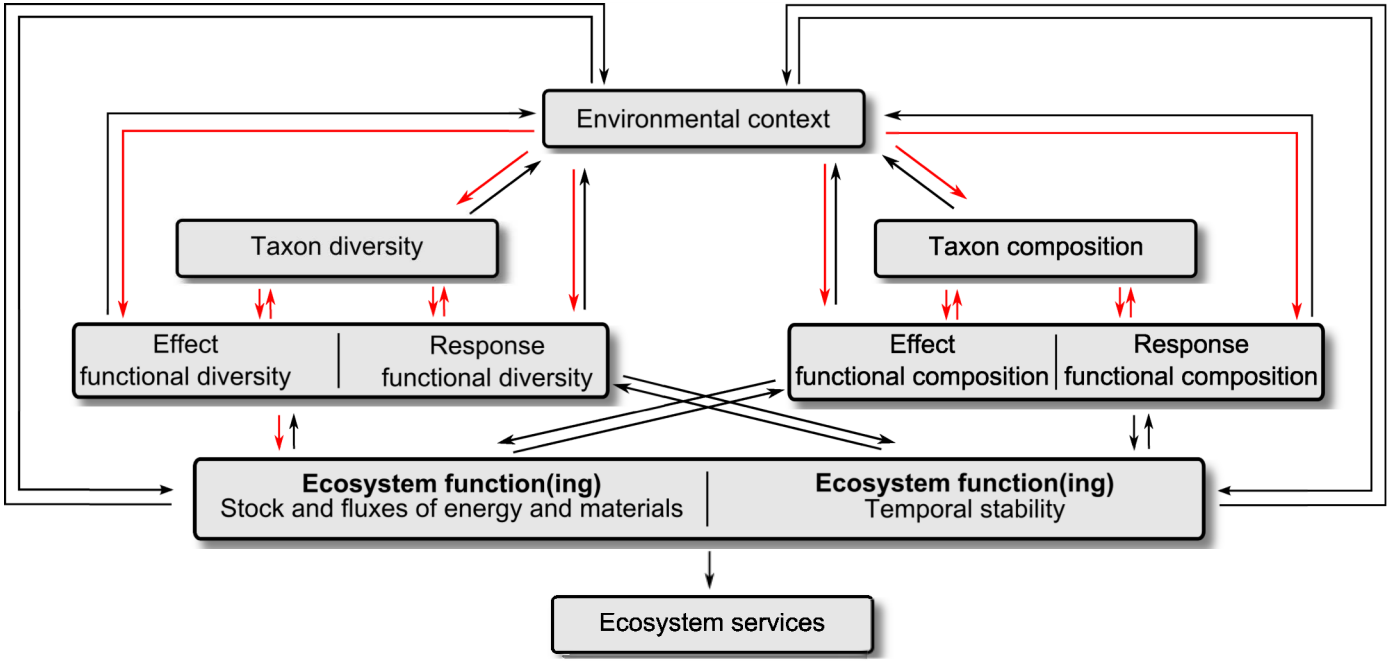

Fig. 3*. Scheme indicating the feedbacks between environmental context, taxon diversity and composition, (effect and response) functional diversity and composition, ecosystem functioning and the provision of ecosystem services. The links considered in this PhD thesis are highlighted in red.*

In the last two decades, many indices have been developed for the quantification of functional diversity (Walker et al. 1999, Petchey and Gaston 2002, Weiher 2011). These functional diversity measures can be categorized according to their dimensionality (i.e. how many traits are involved in their calculation) and the aspect of diversity they entail (richness, evenness, or divergence) (Mason et al. 2005, Weiher 2011). A common way to express functional diversity within a community is the number of functional groups, which comprise functionally similar taxa. However, a major disadvantage of this approach is that taxa within a functional group are considered functionally redundant. There is no single best index to assess functional diversity, but the functional traits to focus on should be those that are relevant to the ecosystem process one wishes to examine (Bengtsson 1998).

In some instances, it is hard to identify or quantify those attributes of the taxa under study that are important to the functioning of the ecosystem. In this case, phylogenetic diversity can be used as a proxy for functional diversity since it usually encompasses most of the functional variation within a community (Cadotte et al. 2011, Srivastava et al. 2012). When used in concert with functional diversity measures, phylogenetic diversity was demonstrated to relate more strongly to ecosystem functioning (Cadotte et al. 2008, 2009). Importantly, the utility of phylogenetic diversity as a measure for functional diversity depends on the strength of phylogenetic signals for functionally important traits and biotic interactions (Srivastava et al. 2012).

It is important to realize that functionally important traits may not only differ between individuals belonging to different taxa (i.e. interspecific variation), but also between individuals within the same taxon (intraspecific variation) (Bolnick et al. 2011). Remarkably, functional diversity within a given taxon may even rival that between different taxa, which calls for a shift from a taxon-based to a trait-based approach (Reiss et al. 2009, Messier et al. 2010).

5 Biodiversity and its effect on ecosystem functioning

There is an ongoing, worldwide decline in marine and terrestrial biodiversity driven by human activities like overexploitation (e.g. fisheries, wood harvesting), habitat degradation (e.g. fisheries, mining of minerals), pollution, eutrophication, and finally climate change (resulting in marine hypoxia, ocean acidification and seawater warming, amongst others) (Chapin et al. 2000, Thiel 2003, Widdicombe and Somerfield 2012). We should care not only from an aesthetic or ethical viewpoint, but also because of the many services ecosystems, and their biodiversity, including the deep sea, provide.

Ecosystem functions comprise three classes: (1) the stock of energy and materials (e.g. biomass), (2) the fluxes of energy and materials (e.g. oxygen production, nutrient cycling, burial of organic matter), and (3) temporal stability (Paterson et al. 2012). Strictly speaking, biodiversity can also be considered as an ecosystem function since it represents the source of future biodiversity. However, in this study, we will not regard diversity as an ecosystem function. The various ecosystem functions govern the functioning of a given ecosystem. Ecosystem services are defined as the processes and conditions of ecosystems that benefit humankind directly or indirectly (Costanza et al. 1997, Beaumont et al. 2007) (Fig. 3). Examples of marine ecosystem services include provision of food and raw materials, gas and climate regulation, recreation, and so on.

As a consequence of the "biodiversity crisis", there has been an explosion of research addressing the influence of biodiversity on ecosystem functioning in the last two decades. Most work has been done on terrestrial systems (Naeem and Wright 2003, Balvanera et al. 2006), but given the many disparities with marine ecosystems (Giller et al. 2004), caution is warranted when extrapolating these results to marine ecosystems. Nevertheless, in the last couple of years, a growing amount of biodiversity-function research has been devoted to the marine environment (Stachowicz et al. 2007). Only recently the biodiversity-ecosystem function relation has been explored in deep-sea sediments based on nematode diversity data from multiple oceanographic regions (Danovaro et al. 2008a).

In terrestrial and marine environments, positive, negative and no diversity effects on ecosystem processes have been recorded, but a positive diversity-function relation has been observed most frequently (Balvanera et al. 2006, Stachowicz et al. 2007). The exact form of the diversity-function relationship, whether positive, negative or non-existent, depends on the metrics that are used to quantify diversity and function (Naeem et al. 1995, Bolam et al. 2002, Balvanera et al. 2006). It is also determined by the spatial scale considered (Bengtsson et al. 2002, Bond and Chase 2002) and the environmental context (Cardinale et al. 2000, Loreau 2000, Hiddink et al. 2009). Although hitherto most studies have investigated the response of ecosystem functioning to changes in biodiversity, we need to move beyond simple unidirectional relationships between biodiversity and ecosystem function and start considering feedbacks between diversity, environmental factors, and ecosystem functioning (Worm and Duffy 2003, Gamfeldt and Hillebrand 2008).

6 Diversity and function of deep-sea nematodes

Marine benthic standing stock declines exponentially with increasing water depth as a result of the diminishing nutrient input from the euphotic layers of the oceans (Wei et al. 2010). However, this reduction in abundance and biomass with increasing water depth is less pronounced for the meiofauna (0.032 – 1 mm) than for the larger macro- and megafauna (Rex et al. 2006, Wei et al. 2010). Deep-sea and shallow-water marine meiofauna are nearly always dominated by the phylum of the nematodes (Heip et al. 1985). Nematode communities in deep-sea sediments show high species and genus diversity at the local scale (i.e. in one sample or set of replicate samples) (Soetaert et al. 1991, Lambshead and Boucher 2003). A considerable fraction of this high local (α) genus diversity is attributable to the presence of many rare genera. However, typical deep-sea genera (e.g. *Acantholaimus*, *Halalaimus* and *Daptonema*) have a cosmopolitan distribution (Vanreusel et al. 2010b), and thus turnover (β) diversity, and consequently regional or global diversity may be moderate compared to local diversity. In addition, most deep-sea genera also occur in shallow marine habitats (Soetaert and Vincx 1987, De Mesel et al. 2006, Fonseca and Decraemer 2008), though generally the species composition is distinct between these two environments (Fonseca et al. 2006b). Only occasionally a new nematode genus is reported from deep-sea samples (Bussau and Vopel 1999, Fonseca et al. 2006a, Bezerra et al. submitted). The low turnover diversity of nematode genera in the deep sea may, however, not apply at the species level (De Mesel et al. 2006, Fonseca et al. 2006a, Danovaro et al. 2009a; but see Lambshead and Boucher 2003).

Because of their numerical dominance, high metabolic rates, and short generation times, nematodes are presumed to be important in the benthic carbon cycle in coastal sediments (Giere 2009). Whether this applies to the deep sea is unclear since information on the life history characteristics (metabolic rates and generation times, amongst others) of nematodes living in this extreme environment is entirely lacking. Some studies have attributed a considerable fraction of total total respiration in deep-sea sedimentary environments to the meiofauna; reported values are 22 % (Eldridge and Jackson 1993), 39 % (Mahaut et al. 1995) and up to 22 % (Baguley et al. 2008). In contrast, other reports covering the continental shelf and the deep sea (Soetaert et al. 2009) or only the deep sea (Piepenburg et al. 1995, Soetaert et al. 1997, Heip et al. 2001) estimated the contribution of meiofauna to total mineralisation to be more limited, ranging between 4 and 13 %.

The most important functions of the benthos are generally considered to be the direct and indirect effects of their feeding activities through which they may influence nutrient fluxes and stabilize the sediment (Snelgrove 1997). The extent to which nematode feeding behaviour

contributes to these ecosystem processes in the deep sea has not been directly quantified yet. The feeding mode of nematodes is generally inferred from the morphology of their mouthparts, on the basis of which four feeding guilds were delineated, i.e. selective deposit feeders (small and toothless or no buccal cavity; feeding type 1A), non-selective deposit feeders (medium-large, toothless buccal cavity; feeding type 1B), epistrate feeders (small-medium buccal cavity with small tooth or teeth; feeding type 2A) and predators/scavengers (large buccal cavity with large teeth or mandibles; feeding type 2B) (Wieser 1953) (Fig. 4). According to their buccal morphology, the majority of the nematode genera frequently encountered in deep-sea sediments can be classified as deposit feeders (1A + 1B). In addition to deposit feeders, deep-sea nematode assemblages often comprise a considerable fraction of epistrate feeders (2A) (e.g. Vanhove et al. 2004, Sebastian et al. 2007, Ingels et al. 2009). In coastal habitats these nematodes feed on diatoms, but given the paucity or lack of these food items in the deep sea, Moens and Vincx (1997) speculated that in the latter environment epistrate-feeding nematodes use their small teeth to scrape off microbial coatings from sediment particles or mucus threads. Considering that benthic bacteria represent a labile carbon source within the primarily refractory sediment pool and that nematode abundances and bacterial parameters regularly covary (Vanreusel et al. 1995b, Sommer and Pfannkuche 2000, Soltwedel et al. 2005, Hoste et al. 2007), it is plausible that most deep-sea nematodes prey selectively on bacterial cells. In support, the nitrogen isotope values of nematodes at the Porcupine Abyssal Plain indicated that this group did not feed on freshly deposited particulate organic matter (Iken et al. 2001). However, bacterivory was not evidenced in feeding experiments which indicated very little assimilation of bacterial carbon (Guilini et al. 2010, Ingels et al. 2010). Also other food sources like cyanobacteria (Moens et al. 2007) or phytodetritus (Moodley et al. 2002, Witte et al. 2003b, Ingels et al. 2010, 2011a, Gontikaki et al. 2011a) were not taken up in large quantities by deep-sea nematodes. The absence of significant carbon uptake in these experiments suggests that these food sources are not adequate and other dietary items need to be considered.

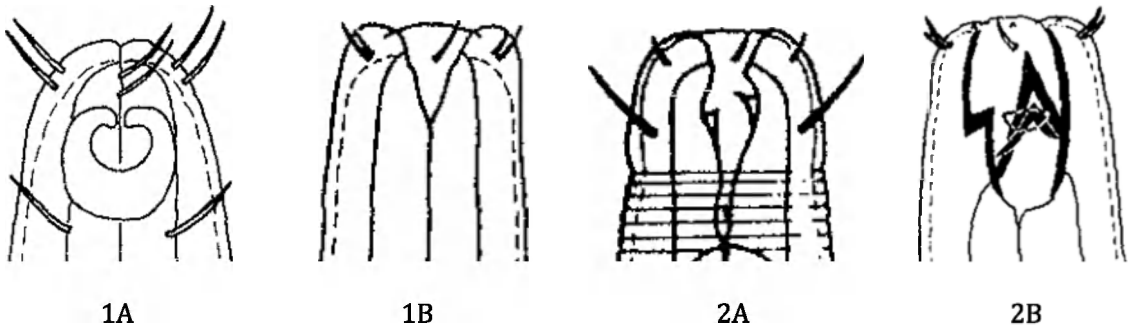

Fig. 4. *Representatives of the nematode feeding guilds according to Wieser (1953). 1A: selective deposit feeder, 1B: non-selective deposit feeder, 2A: epistrate feeder and 2B: predator/scavenger.*

In marine nematode research, the diversity of mouth morphologies or trophic diversity is regularly employed as a measure of functional diversity (e.g. Vanaverbeke et al. 2004, Danovaro et al. 2008a, Gambi et al. 2009, Ingels et al. 2009, 2011b, Schratzberger et al. 2009). This index incorporates the number of feeding guilds and the distribution of individuals among these guilds (Heip et al. 1985), and thus serves as a measure of resource partitioning. Other traits that have been used to quantify nematode functional composition and/or diversity in shallow and deep marine environments are body shape or size and life history characteristics (Schratzberger et al. 2007, Armenteros et al. 2009, Hasemann and Soltwedel 2011). The shape or size of nematodes may relate to mobility, vulnerability to predation or prevailing oxygen levels (Soetaert et al. 2002, Schratzberger et al. 2004). The range of life history characteristics determines how assemblages respond to environmental change; these characteristics have been translated into the Maturity Index, which is used to assess the degree of environmental disturbance (Bongers 1990, Bongers et al. 1991).

7 International framework

7.1 BIOFUN

The EUROCORES-EuroDEEP project BIOFUN ("BIOdiversity and ecosystem FUNctioning in Southern European Deep-sea environments: from viruses to megafauna", 2007-2011) was a collaborative research project involving seven principal (CSIC, Spain; CNR-ISMAR, Italy; NIOO, The Netherlands; NIOZ, The Netherlands; UGent, Belgium; NUIG, Ireland; CEFREM, France) and three associated (DISMar-UNIVPM, Italy; HCMR, Greece; SNG, Germany) European partners. The

overall aim of BIOFUN was to understand the link between biodiversity patterns and ecosystem functioning in relation to environmental conditions along a gradient of increasing oligotrophy, from the Galicia Bank in the northeast Atlantic to the western, central and eastern Mediterranean. This sampling strategy allowed for the assessment of the response of deep-sea ecosystems to changing trophic conditions. BIOFUN was the first project to investigate all food web components, from viruses and microbes to megafauna, including commercially important species. The project was structured in four scientific work packages: (1) physicochemical habitat characteristics; (2) biodiversity and biogeography; (3) ecosystem functioning: food web processes and life history patterns; and (4) linkages between ecosystem functioning and biodiversity: tools for disturbance evaluation. New and essential information for a correct management of the biodiversity and natural resources of the deep sea and for understanding the role of deep-sea biota in global biogeochemical cycles will be provided by the results gathered in this project.

Within the frame of the BIOFUN project, the objectives of the present doctoral research were to (1) characterize nematode diversity in contrasting deep-sea environments, (2) identify the role of nematodes in the benthic carbon flux, and (3) investigate the link between diversity and functioning for nematode assemblages. The data gathered in this work will contribute to three collaborative papers addressing, for all size groups, the shift in community composition from the Atlantic to the Mediterranean, food web structure in contrasting environments and the link between biodiversity and ecosystem functioning.

7.2 HERMES and HERMIONE

The European Commission's 6th Framework Research Project (EC FP6) HERMES ("Hotspot Ecosystem Research on the Margins of European Seas") started in 2005 and came to an end in 2009 (Weaver and Gunn 2009). HERMES was then succeeded by HERMIONE (EC FP7), which stands for "Hotspot Ecosystem Research and Man's Impact on European Seas" and ended in September 2012 (Weaver et al. 2009). Both projects were supported by a large, multidisciplinary consortium, comprising both academic and professional bodies. As such, HERMES and HERMIONE allowed for a fully interdisciplinary approach to continental margin research, with expertise in deep-sea biology, geology, biogeochemistry, microbiology and physical oceanography. Socio-economists and ecological modellers were involved to integrate scientific findings, which was of use to policy makers.

HERMES and HERMIONE focused on so-called biodiversity hotspots along the European margins including cold seeps, canyons, cold-water coral sites, carbonate mounds, anoxic environments

and the slopes adjacent to these habitats. Compared to its predecessor, HERMIONE placed greater emphasis on anthropogenic impacts in the deep-sea environment and the translation of scientific data into policy (Weaver et al. 2009). In addition, even more effort was directed towards public outreach, and new study areas included seamounts and hydrothermal vents. The overall goal of the two projects was to increase our understanding of the diversity, structure, function and dynamics of hotspot margin ecosystems, which should lead to a better management of our continental margins. The present work contributed to HERMES and HERMIONE by investigating the diversity and function of nematodes in HERMES/HERMIONE target areas, i.e. the Gulf of Cádiz, and more specifically the Darwin mud volcano, and the Mediterranean Sea.

8 Study areas

The study areas of the present doctoral research were situated along the continental margins and abyssal basins of southern Europe (Fig. 5). In the following paragraphs, the general oceanographic features and broad biological characteristics of each area are briefly presented.

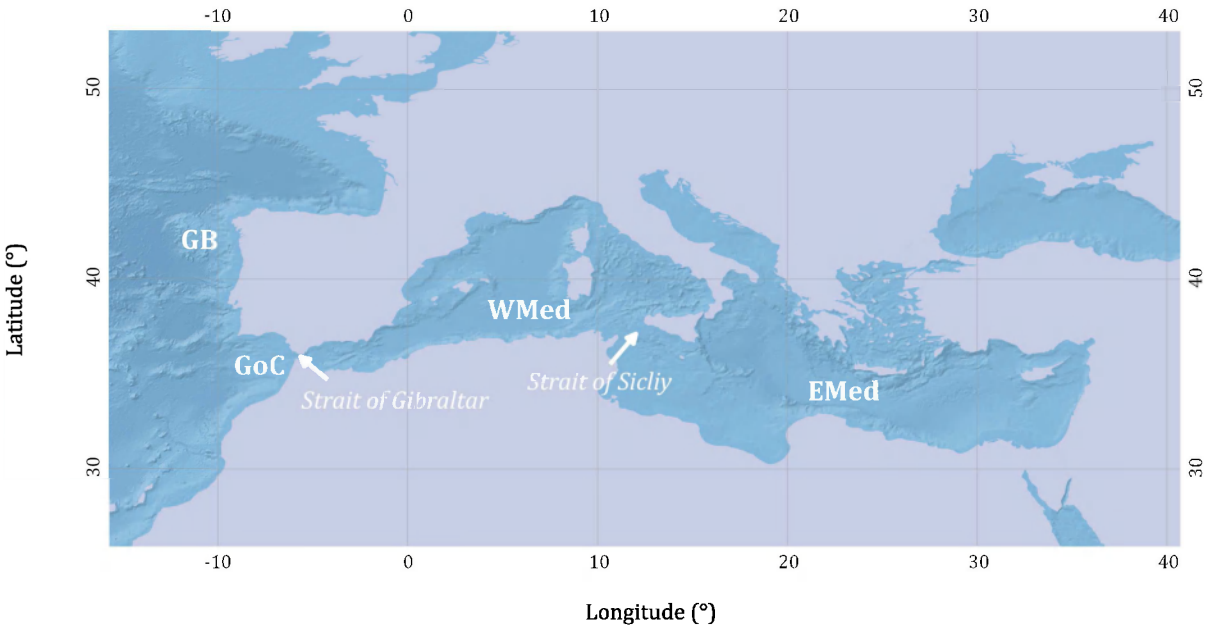

Fig. 5. *Overview map with all study areas. GoC: Gulf of Cádiz, GB: Galicia Bank, WMed: western Mediterranean, EMed: eastern Mediterranean. The Strait of Gibraltar separates the deep Mediterranean from the deep Atlantic, while the Strait of Sicily forms the border between the western and eastern deep Mediterranean basins.*

8.1 The Darwin mud volcano in the Gulf of Cádiz

The Gulf of Cádiz is located in the northeast Atlantic at the boundary between the Iberian and African continental plate, westwards of the Strait of Gibraltar (Fig. 5). The area is under the influence of surface Atlantic inflow and intermediate depth (~1200 m) Mediterranean outflow water. In the Gulf of Cádiz, the latter entrains North Atlantic Central Water and nutrient-rich Antarctic Intermediate Water, after which it sinks and flows northward as the Mediterranean undercurrent (Van Aken 2000, Louarn and Morin 2011).

The Gulf of Cádiz has a complicated geological history, and experienced several phases of rifting, convergence and strike-slip motions (Maldonado et al. 1999). The centre of the area is occupied by an accretionary wedge, the so-called Olisostrome, which was formed through the westward migration of the Gibraltar Arc orogenic belt. Seepage in the Gulf of Cádiz is closely associated with this accretionary wedge-type setting, which is related to the convergence of the African and Eurasian plates. The first fluid venting features were identified in 1996 (Baraza and Ercilla 1996), and from then onwards, subsequent expeditions have discovered many other fluid escape structures like mud volcanoes and methane-related authigenic carbonates (Pinheiro et al. 2003, Somoza et al. 2003, Van Rensbergen et al. 2005, León et al. 2007). So far, around 50 mud volcanoes have been identified which all exhibit low to moderate seepage activity (Niemann et al. 2006, Stadnitskaia et al. 2008). Several mud volcanoes in the Gulf of Cádiz have been investigated for their microfauna (Niemann et al. 2006) or macrofauna (Ravara et al. 2007, Genio et al. 2008, Hilario 2008, Rodrigues et al. 2008, 2011, Sommer et al. 2009, Hilario et al. 2010), whilst the meiofauna has been disregarded.

The Darwin mud volcano (35° 23.51' N, 7° 11.48' W) was discovered in 2006 during the RSS Charles Darwin Cruise nr. 178 (Masson and Berndt 2006). The summit of this mud volcano is covered by a thick carbonate crust, indicative of past high seepage activity. At present, the mud volcano is in a near dormant stage and seepage is limited to a sediment-covered area in the north-western part of the summit (Vanneste et al. 2012). Atop of the mud volcano, countless, but mostly empty, *Bathymodiolus mauritanicus* shells are found (Genio et al. 2008) and living specimens only occur along cracks in the crust. There are no dense aggregations of living chemosynthetic megafauna associated with the seep sediments; non-chemosynthetic megafauna comprises scavenging crabs, and corals attached to the carbonate crust (Vanreusel et al. 2009).

8.2 The Mediterranean Sea

The Mediterranean Sea is the largest (3.0 million km^2) and deepest (average depth: ± 1500 m) semi-enclosed basin in the world. Because the continental shelves are quite narrow, most of the Mediterranean waters are below 200 m water depth and thus can be classified as deep sea. The Mediterranean Sea consists of several sub-basins, which can be divided into a western and eastern set divided by the shallow Strait of Sicily (Figs. 5 and 6). This thesis discusses environmental conditions and nematode community characteristics of sediments sampled in the Algerian and Algero-Provençal basin (i.e. western Mediterranean), and in the the south Ionian and the north Levantine Sea (i.e. eastern Mediterranean). The western part of the Mediterranean is coupled with the Atlantic Ocean through the Strait of Gibraltar, while the eastern part connects with the Black Sea through the strait of Bosporus and the Dardanelles. In the south-east, the basin links with the Red Sea through the Suez Canal. The Mediterranean is characterized by some unusual oceanographic features (Tyler 2003, Sardà et al. 2004). Because of the continental climate, evaporation exceeds freshwater input giving rise to highly saline waters (37.5-39.5 psu). Furthermore, the Mediterranean deep waters are distinguished by an unusually high temperature of 13 °C, which remains fairly constant with increasing water depth.

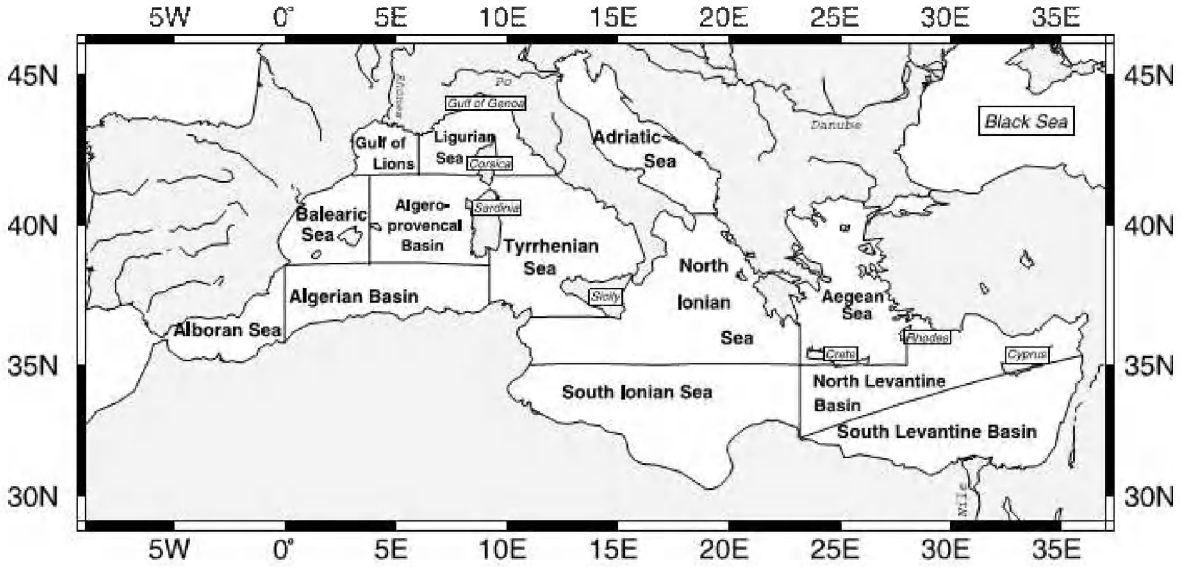

Fig. 6. *Overview of the various sub-basins in the Mediterranean Sea. From Bosc et al. (2004).*

The Mediterranean Sea displays complex thermohaline circulation patterns (Bergamasco and Malanotte-Rizzoli 2010) (Fig. 7). The freshwater deficit pushes relatively fresh surface Atlantic water through the Strait of Gibraltar into the Mediterranean. Seawater temperature and hence

evaporation are higher in the east compared to the west, and consequently the difference in water level drives the body of Atlantic surface water from west to east. Along its path, the Atlantic water body becomes warmer and saltier (Modified Atlantic Water or MAW). In the Levantine Basin, winter cooling increases the density of the Atlantic Water, where it eventually sinks to about 400 m depth, forming Levantine Intermediate Water (LIW). LIW circulates back to the west across the shallow Strait of Sicily, and finally back into the Atlantic as Mediterranean Outflow Water (MOW). This MOW reaches relatively far into the North Atlantic, where it contributes to dense water formation in the Norwegian Sea. In addition to this open, thermohaline circulation cell comprising the entire Mediterranean basin, deep water formation takes place during winter in the eastern and western basin. In the western Mediterranean, dense water is formed in winter in the Gulf of Lions (Western Mediterranean Deep Water or WMDW), whereas in the eastern basin, Cretan Sea Overflow Water and to a lesser extent Adriatic Deep Water comprise the body of Eastern Mediterranean Deep Water (EMDW).

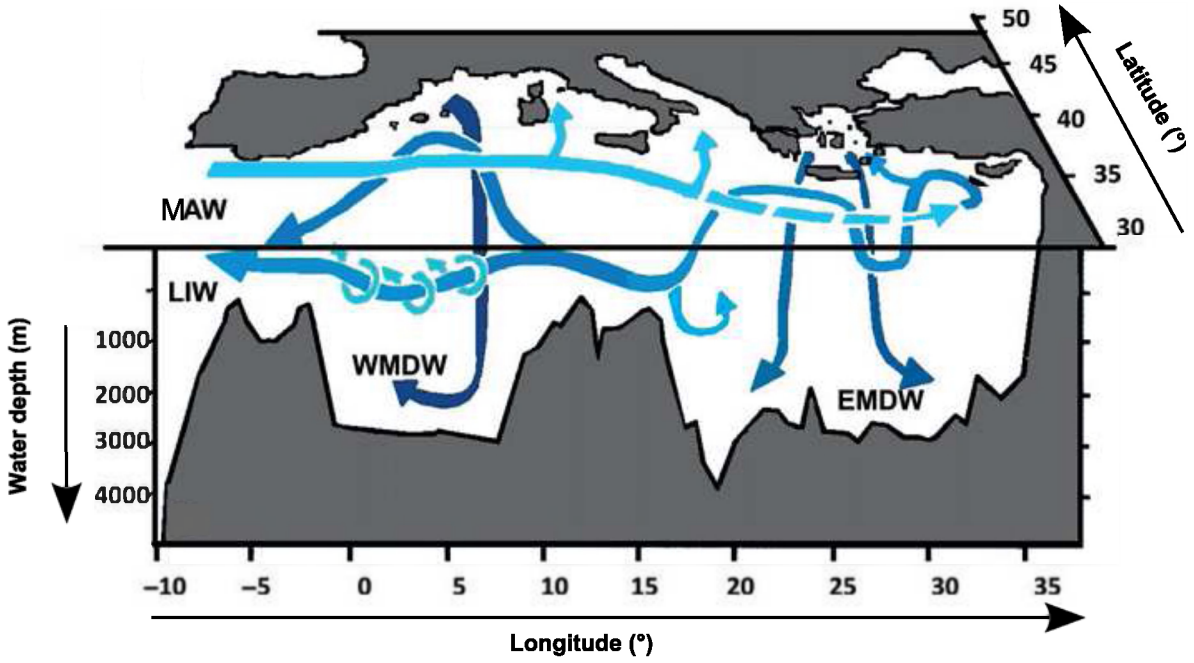

Fig. 7. *Scheme of the thermohaline circulation in the Mediterranean. MAW: Modified Atlantic Water, LIW: Levantine Intermediate Water, EMDW: Eastern Mediterranean Deep Water, WMDW: Western Mediterranean Deep Water. Slightly modified from Bergamasco and Malanotte-Rizzoli (2010).*

The Mediterranean deep sea is one of the most oligotrophic regions in the world. In addition, the Sea is characterized by a decline in primary productivity along its longitudinal axis (Danovaro et

al. 1999a, Turley et al. 2000, Siokou-Frangou et al. 2010). This gradient in productivity is caused by a greater nutrient input in the west (owing to Atlantic water inflow and river discharge) compared to the east. Nevertheless, there is substantial regional variability in primary productivity in the western and eastern basin due to local hydrological features (Estrada 1996, Bosc et al. 2004). In the western part, hotspots of primary productivity are situated in the Alboran Sea and along the north-western margin in the Gulf of Lions. In the eastern basin, the Aegean and the Adriatic Sea are characterized by the highest levels of primary production, strongly contrasting with the highly oligotrophic Levantine basin. Apart from spatial variation, there is considerable seasonality in primary productivity with a general peak in winter-early spring and a minimum in summer (Bosc et al. 2004, D'Ortenzio and Ribera d'Alcalà 2009).

Primarily owing to the low surface productivity, the benthos in the deep Mediterranean is impoverished compared to other oceanographic regions (Sardà et al. 2004). The megafauna in the deep Mediterranean is dominated by fish and decapod crustaceans (Tecchio et al. 2011), which stands in stark contrast with the Atlantic Ocean where holothurians prevail (e.g. Billett et al. 2001). Quantitative data on the deep Mediterranean macrofauna are extremely scarce (Sardà et al. 2004). In the western Mediterranean, at the Catalan Sea slope, polychaetes were reported as the predominant taxon in the Bésos canyon, whereas Sipuncula and Bivalvia dominate the adjacent slope sediments (Mamouridis et al. 2011). The macrofauna in the eastern Mediterranean basin mainly constitutes of polychaetes (Tselepides et al. 2000, Kröncke et al. 2003).

Although the Mediterranean Sea constitutes less than 1 % of the world ocean's surface, this basin hosts more than 7.5 % of global marine biodiversity (Bianchi and Morri 2000). It is considered a biodiversity hotspot, with an estimated total amount of (at least) 17000 species (Coll et al. 2010). The complex geological history (e.g. the Messinian Salinity Crisis; Krijgsman et al. 1999) and the variety in environmental conditions have been suggested as potential drivers for this high diversity. However, only a small fraction of this Mediterranean diversity pertains to deep-sea habitats, since they are largely under-sampled. Danovaro et al. (2010) estimated total Mediterranean deep-sea diversity (excluding prokaryotes) at 2805 species, of which 66 % was undescribed. These authors predicted that most species yet to be described belong to the phylum of the Nematoda. Major threats to (deep-sea) Mediterranean biodiversity include pollution, overexploitation and the concomitant habitat degradation (although there is ban of trawling activities beneath 1000 m water depth), and climate change (Bianchi and Morri 2000, Coll et al. 2010, Danovaro et al. 2010).

8.3 The Galicia Bank region

The Galicia Bank is a seamount located about 200 km off the north-western coast of Spain, on the distal edge of the north-western Iberian margin. It is separated from the shallower parts of the continental margin by a 3000 m deep channel, the Galicia Interior Basin. The seamount is part of the Galicia Bank region, which forms a topographic bulge with an overall mountainous shape and which consists of different morphostructural provinces (Vazquez et al. 2008, Ercilla et al. 2011). The region is under influence of different water masses of north Atlantic and Mediterranean origin (Fiúza et al. 1998). Mediterranean Outflow Water constitutes the intermediate water layer (600-1600 m), which bifurcates at the Galicia Bank, with one branch flowing to the west of the seamount and the other branch flowing northwards along the continental slope (Mazé et al. 1997). The Galicia Bank region has mainly been studied for its geological aspects and has gained scientific attention since the sinking of the Prestige oil tanker in 2002 (Ercilla et al. 2006, Vazquez et al. 2008).

The Galicia Bank seamount has a relatively flat top, which is covered by a thick layer of foraminiferal ooze (Duineveld et al. 2004). The granulometric characteristics of the surficial sediments at the Galicia Bank (high median grain size and carbonate content) were ascribed to the high current velocities, which transport finer particles as bedload (Van Weering et al. 2002). Megafauna sighted at the Galicia Bank include isolated patches of cold-water corals (*Lophelia pertusa*, *Madrepora oculata*), found around 800 m water depth, and numerous mobile crinoids (Duineveld et al. 2004). Macrofaunal densities and biomass were reported to be low, which was ascribed to the low carbon deposition, as a consequence of the strong hydrodynamic regime (Flach et al. 2002). The only investigation involving meiobenthos on the Galicia Bank so far, addressed higher meiofaunal taxon composition at one station at 770 m water depth at the Galicia Bank summit (Flach et al. 2002).

Overall, the Iberian margin is characterized by an average primary productivity of 220 g C m^{-2} yr^{-1} (Joint et al. 2002) which is governed by intense, wind-driven upwelling during spring and summer (McClain et al. 1986). Filaments of upwelled waters can protrude up to 200-300 km offshore (McClain et al. 1986, Joint et al. 2002).

9 Aims and outline of the thesis

This PhD research intended to gain more insight into the drivers of nematode diversity at different spatial scales and in distinct environmental settings, and how this impacts their

functioning within deep-sea ecosystems through field observational studies (Chapters 2, 4 and 5) and experiments (Chapter 3) carried out in several southern European deep-sea localities. The environmental drivers of nematode taxon and functional structure and diversity in the deep sea were investigated in both a cold seep system in the Gulf of Cádiz (Darwin mud volcano, **Chapter 2**) as well in sediments showing no signs of chemosynthesis (in the Galicia Bank region, northeast Atlantic, and in the Mediterranean, **Chapters 4 and 5**). Apart from the general introduction (Chapter 1) and discussion (Chapter 6), the chapters of this thesis represent stand-alone research articles which were either published, or are currently under review. Consequently, there inevitably is some degree of overlap between the different chapters, particularly in the introduction and materials and methods sections.

In the first study (**Chapter 2**), we evaluate the small-scale (~meters) variability in nematode diversity and community composition at the low-activity Darwin mud volcano in the Gulf of Cádiz in relation to pore-water geochemical conditions along a 10-m transect from a seep site at the summit towards the slope of the mud volcano. In addition, dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) and buccal morphology were used to infer dietary preferences under varying seepage regimes. The genera that prevailed in the seep sediments were subjected to electron microscopy to look for the presence of symbiotic bacteria or detoxification structures, which would explain their tolerance of the harsh local environmental conditions. This work was published as *Pape E, Bezerra TN, Vanneste H, Heeschen K, Moodley L, Leroux F, Van Breugel P, and Vanreusel A (2011) Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz) Marine Ecology Progress Series 438: 71-83.*

Chapter 3 presents the results of a set of onboard experiments during which sediment cores from different deep-sea environments (i.e. the Galicia Bank seamount and adjacent continental slope, and a slope in the western Mediterranean) were injected with $\delta^{13}C$ labeled dissolved organic matter (DOM) to unravel nematode feeding strategies and to assess the potential fate of DOM carbon. Slope sediments were injected with glucose ("simple" DOM) or "complex" diatom-derived DOM to investigate the influence of the DOM composition on carbon assimilation patterns. We investigated whether nematode ^{13}C label assimilation resulted from either direct DOM ingestion or from preying on ^{13}C labeled bacteria by evaluating their contribution to the diet under both hypothetical feeding strategies. The flow pathways of DOM carbon through the bacteria and nematodes were reconstructed on the basis of the assimilation rates observed in the feeding experiments and theoretical estimates of assimilation and respiration efficiencies. The manuscript describing these experimental results was accepted with moderate revision by Deep-Sea Research I (*Pape E, van Oevelen D, Moodley L, Soetaert K, and Vanreusel A. Nematode*

feeding strategies and the fate of dissolved organic matter carbon in different deep-sea environments).

Where Chapter 2 deals with small-scale variations in the structure of nematode assemblages at a cold seep, **Chapter 4** aims at studying the regional (100s of kilometres) variability in environmental factors and nematode community structure in photosynthesis-dependent sediments. The primary aim of this chapter is to determine if and how nematode assemblages in bathyal and abyssal sediments are affected by variations in surface primary productivity. Along a west-to-east axis spanning the Galicia Bank region and the entire Mediterranean basin, we verified the presence of a presumed trophic gradient in the pelagic (using satellite based measurements of primary productivity and sedimentation rates) and benthic (phytopigment concentrations, carbon and nitrogen content) zone. We evaluated the effect of this cline in food quantity and quality on standing stock, individual biomass, and on taxon and trophic structure of nematode communities. In addition, we assessed the importance of both organic matter deposition and benthic environmental characteristics for the distribution and structure of nematode communities. The paper discussing these results is currently in press in PLoS One as *Pape E, Jones DOB, Manini E, Bezerra TN, and Vanreusel A. Benthic-pelagic coupling: effects on nematode communities along southern European continental margins.*

Undoubtedly, nematode diversity in photosynthesis-dependent sediments is driven by other environmental variables than that in sediments affected by chemosynthesis (studied in Chapter 2). In **Chapter 5**, the environmental drivers of deep-sea nematode taxon and functional diversity are investigated along the same productivity gradient that was considered in the previous chapter. It was already mentioned that the biodiversity crisis has resulted in a surge of studies addressing the link between biodiversity and ecosystem functioning (Hooper et al. 2005, Balvanera et al. 2006, Stachowicz et al. 2007, Solan et al. 2012) but this type of research is seldom undertaken in the deep sea (Danovaro et al. 2008a). The comparison of carbon assimilation rates between different environments studied in Chapter 3 hints on how community composition and environmental conditions may influence carbon processing rates. In Chapter 5, we investigate the direct link between nematode taxon and functional diversity and nematode carbon mineralisation, inferred from biomass-specific respiration rates. This chapter was submitted for publication in a special Biogeosciences issue on the HERMIONE project entitled "Deep ecosystems of the European Seas" as *Pape E, Bezerra TN, Jones DOB, and Vanreusel A. Unravelling the environmental drivers of deep-sea nematode biodiversity and its relation with carbon remineralisation along a longitudinal primary productivity gradient.* The manuscript has been published as a discussion paper on the Biogeosciences Discussions (BGD) website and is currently under review for Biogeosciences.

Finally, **Chapter 6** integrates the results of the preceding scientific chapters, and provides guidelines for future research.


Dave McCarthy

Chapter 2

Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz)

Modified from the following publication:

Pape E, Bezerra TN, Vanneste H, Heeschen K, Moodley L, Leroux F, van Breugel P, and Vanreusel A (2011) Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz). Marine Ecology Progress Series 438:71–83

ABSTRACT – We sampled the Darwin mud volcano (MV) for meiofaunal community and trophic structure in relation to pore-water geochemistry along a 10 m transect from a seep site on the rim of the crater towards the MV slope. Pore-water profiles indicated considerable variation in upward methane (CH_4) flow among sediment cores taken along the transect, with highest flux in the seep sediment core, gradually decreasing along the transect, to no CH_4 flux in the core taken at a 5 m distance. Low sulphate concentrations and high levels of total alkalinity and sulphide (H_2S) suggested that anaerobic oxidation of methane (AOM) occurred close to the sediment surface in the seep sediment core. High H_2S levels had a genus- and species-specific impact on meiofaunal densities. Nematode genus composition varied gradually between sediment cores, with the genus *Sabatieria* dominating almost all sediment cores. Genus diversity increased with increasing distance from the seep site. These limited data suggest that the community structure of seep meiofauna is highly dependent on local (a)biotic habitat characteristics, and a typical seep meiofaunal community cannot be delineated. Stable isotope values suggested the nematode diet up to 10 m from the seep site included thiotrophic carbon. The thicker hemipelagic sediment layer (photosynthetic carbon), the increased trophic diversity, and the heavier nematode $\delta^{13}C$ farther from the seep site point to a decrease in thiotrophy and an increase in photosynthetic carbon in the nematode diet.

1 Introduction

Mud volcanoes (MVs), geological structures driven by fluid flow, are characterized by a high patchiness of biochemical and physical characteristics. Fluid-flow rates, pore-water concentrations of hydrogen sulphide (H_2S) and methane (CH_4), and the thickness of the hemipelagic sediment veneer, on top of the reduced sediments, can change rapidly over short distances (meters to centimetres) (Levin et al. 2003). The heterogeneity in these properties is the main parameter driving the distribution of macro- and megafauna at seeps (Levin 2005), resulting in patches of tubeworm clusters, mussels or clams, and bacterial mats or bare reduced sediments. Meiofauna can also vary on a scale of meters in terms of taxonomic composition and biodiversity in relation to sediment biogeochemistry (Van Gaever et al. 2009c).

There is no consistent response of meiofauna to seep conditions. Meiofaunal densities at different deep-sea seeps are higher (Olu et al. 1997, Van Gaever et al. 2006) or similar (Shirayama and Ohta 1990) compared to non-seep sediments. In seep environments, nematodes usually are the predominant metazoans, although sometimes copepods dominate (Van Gaever et al. 2006). Generally, deep-sea nematodes are characterized by high local diversity (Lambshead and Boucher 2003). Cold seeps, however, exhibit substantially reduced species diversity, harbouring only a few dominant species (Levin 2005, Vanreusel et al. 2010a). The low diversity in these habitats has been attributed to the harsh abiotic conditions, created by the high H_2S and low oxygen levels (Levin 2005).

Besides high biogeochemical and physical heterogeneity, seeps differ from most deep-sea environments in the local production of organic matter through chemosynthesis. Consequently, possible food sources for seep fauna, including meiobenthos are (1) organic matter derived from symbiotic chemoautotrophic bacteria and (2) free-living chemoautotrophic bacteria, in addition to (3) photosynthetic organic matter, delivered to all deep-sea habitats. Studies on the diet of seep meiofauna are few. Both Van Gaever et al. (2006, 2009b) and Spies and DesMarais (1983) found seep nematodes to be feeding on free-living sulphur-oxidising bacteria. To date, there is no evidence of symbioses between nematodes and chemosynthetic bacteria at deep-sea seeps (Vanreusel et al. 2010a), and observations of symbionts associated with seep nematodes are restricted to shallow waters (Dando et al. 1991, Ott et al. 2004).

The present study examined the community structure and feeding ecology of the meiofauna, with a focus on nematodes, at a MV in the Gulf of Cádiz, which we then related to geochemical gradients along a 10 m transect from a seep site towards nearby hemipelagic surface sediments, and 2 sites farther away from seep influence. Our study differs from previous analyses on seep

meiofauna, because it concerns isolated seep sediments situated on a low-activity MV. We addressed the following questions:

- Does pore-water composition influence horizontal and vertical distribution of meiofauna on a small scale?
- Are the seep sediments colonized by a specialized community that differs from the hemipelagic sediments in density, biomass and taxonomic composition (genera and species)?
- What is the nematode diet inferred from stable isotope analyses and buccal morphology? Do seep conditions influence nematode trophic diversity?

2 Materials and methods

2.1 Study area

The Gulf of Cádiz (34°- 37°15' N, 9° - 6°45' W) is a tectonically active region west of the Strait of Gibraltar, encompassing the boundary between the European and African plates. It is one of the largest cold-seep areas on the European margins with > 30 MVs between 200 and 4000 m deep (Pinheiro et al. 2003, Somoza et al. 2003, Van Rensbergen et al. 2005).

The summit (1100 m depth) of the Darwin MV (35°23.51' N 7°11.48'W; Fig. 1A) is covered with a large, fractured carbonate crust. At the time of sampling, countless, but mostly empty, *Bathymodiolus mauritanicus* shells covered the MV top (Genio et al. 2008). Living specimens were only present in small clumps along cracks in the crust. When disturbed by the remotely operated vehicle (ROV) temperature probe, a small area of dark-coloured sediment (ca. 100 cm^2), from here on referred to as "seep site", emitted gas. Small carbonate blocks and white sediments, indicative of bacterial activity, which covered hard substrate, surrounded the seep site (Vanreusel et al. 2009). No dense aggregations of living chemosynthetic megafauna were associated with the seep sediments or 2 m away. At the MV centre, megafauna comprised scavenging crabs and corals attached to the carbonate crust.

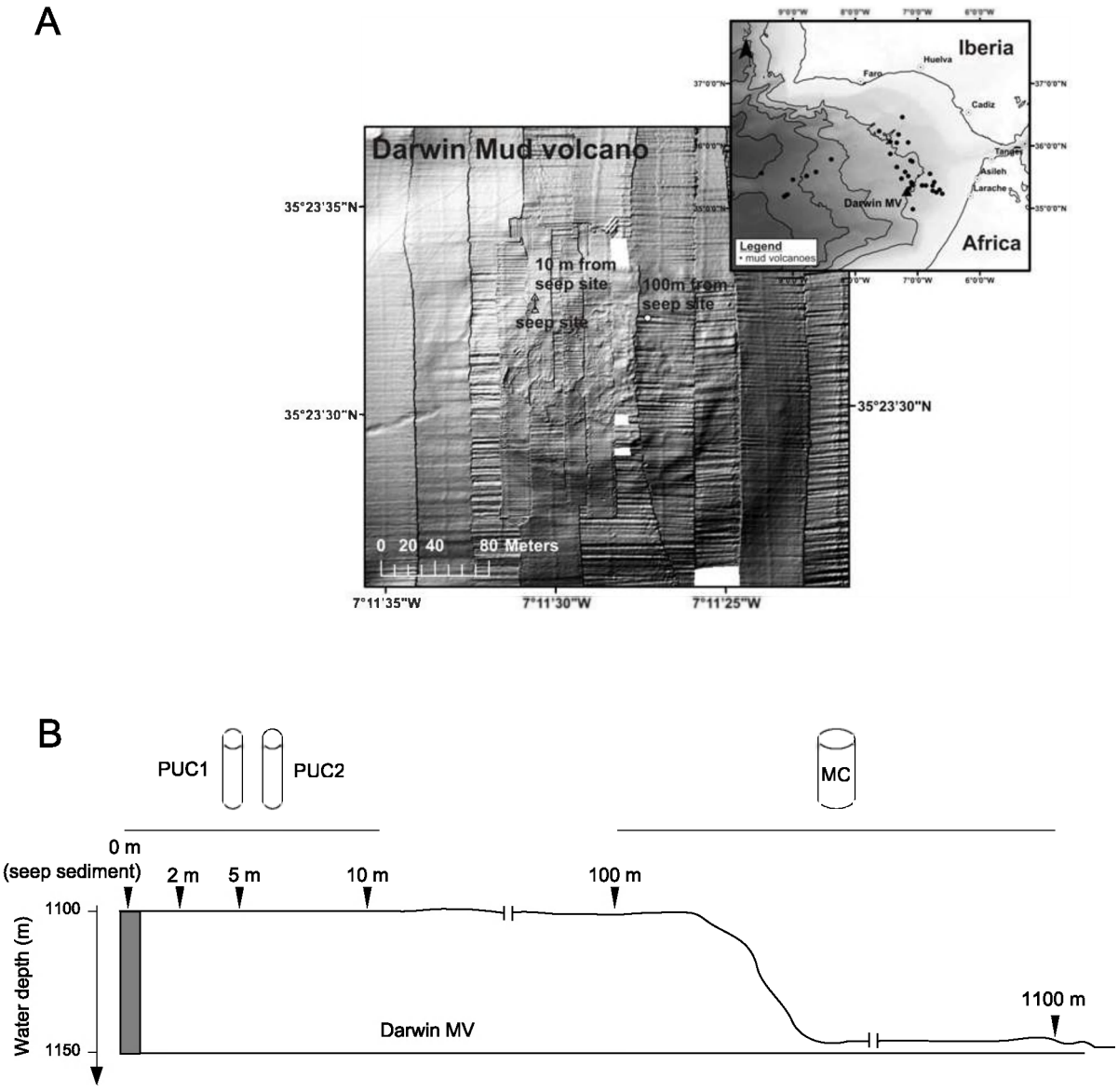

Fig. 1. *Darwin mud volcano (MV). (A) Bathymetric map with the core locations on the MV indicated (courtesy of the National Oceanography Centre Southampton) (B) Schematic representation of the sampling strategy. PUC1: Push Core 1, sampled for pore-water geochemistry and meiofaunal community analyses; PUC2: Push Core 2, sampled for pore-water CH_4 concentration, porosity and nematode stable isotope signatures; MC: megacorer sample for meiofaunal community analyses.*

2.2 Sampling strategy

Sediment cores were collected during the JC10 expedition to the Gulf of Cádiz in May 2007 onboard the RRS *James Cook* (Table 1). We were unable to collect replicate samples because of the high heterogeneity of the habitat and the small size of the seep site. However, the present

study is the first to identify potential interactions between seep meiofauna and pore-water geochemistry measured at such a small spatial scale.

Table 1. *Sediment cores in relation to distance from the Darwin mud volcano seep site. PUC: push core, MC: megacore. 0 = seep sediment. PUC1: meiofaunal community structure and pore-water geochemistry. PUC2: porosity, pore-water CH_4 concentration and nematode stable isotopes. MC: Meiofaunal community structure.*

Distance from seep site (m)	Position	Height hemipelagic layer (% core length)	Gear	No. of cores	Analyses
0 (seep sediment)	35°23.539'N, 7°11.508'W	0	PUC	2	PUC1
					PUC2
2	35°23.543'N, 7°11.506'W	22-33	PUC	2	PUC1
					PUC2
5	35°23.543'N, 7°11.509'W	53-63	PUC	2	PUC1
					PUC2
10	35°23.547'N, 7°11.511'W	71	PUC	2	PUC1
					PUC2
100	35°23.537'N, 7°11.454'W	100	MC	1	MC
1100	35°23.965'N, 7°11.121'W	100	MC	1	MC

Using the ROV "Isis", we collected 2 push cores (PUCs, 25.5 cm^2) at each of the 4 sites along a 10 m transect between the seep site and an area with a considerably thicker hemipelagic sediment layer (Fig. 1; Table 1). One PUC was taken with a core-liner, with openings every 2 cm, to extract pore-water using Rhizons (Seeberg-Elverfeldt et al. 2005). These pore-waters were sub-sampled on board for nutrient and anion analysis. The top 0-5 cm of the sediment in the first PUC was sliced horizontally per cm. The second PUC was sub-sampled for CH_4 and porosity analyses, and we stored the remaining sediment in 2 cm slices at -30 °C for stable isotope analysis. Besides the 10 m transect, we sampled 2 sites at ~ 100 (on the MV) and ~ 1100 m from the seep site (off the MV) with a megacorer (75.4 cm^2). These samples were exclusively analyzed for meiofaunal community structure.

2.3 Pore-water geochemical analyses

Total alkalinity (TA) and hydrogen sulphide (H_2S) were measured immediately after pore-water extraction: TA by titrating against 0.05 M HCl while bubbling nitrogen through the sample (Ivanenkov and Lyakhin 1978) and H_2S using standard photometric procedures (Grasshoff et al. 1999) adapted for pore-waters with high H_2S levels (magnitude of mM). Concentrations of all other species were analyzed at the National Oceanography Centre Southampton (NOCS). Sulphate (SO_4^{2-}) was measured by ion chromatography (Dionex ICS2500), with reproducibility > 1.5 % (determined by repeat analysis of a seawater standard as well as single anion standards). We measured dissolved CH_4 in sediment samples taken immediately after opening the cores according to the headspace vial method (Reeburgh 2007). An aliquot of sediment (~ 3 cm^3) was withdrawn, placed in a glass vial, and 5 ml of 1M NaOH was added to prevent further microbial activity (Hoehler et al. 2000). The vial was crimped shut, and the sample shaken vigorously to release the gases. CH_4 concentration in the headspace was determined by gas chromatography (Agilent 6850) at the NOCS. These headspace CH_4 measurements were then converted to dissolved CH_4 concentrations following Hoehler et al. (2000). Depressurization and warming of the cores during sediment retrieval is likely to have led to degassing, so concentrations of CH_4 (which is generally oversaturated in pore-waters) and H_2S represent minimum values. Therefore, profiles were compared relative to one another, rather than to measurements in other studies.

2.4 Meiofaunal community analyses

We washed the formalin-fixed samples over a 32 µm mesh sieve and extracted the meiofauna from the sediment by Ludox centrifugation (Heip et al. 1985). Meiofauna was then sorted, enumerated and identified at higher taxonomic level. Where possible, ca. 100 nematodes were picked per 1 cm slice and identified to genus level. *Sabatieria*, the dominant genus in all cores but one, was identified to species. Additionally, we measured length (µm) and maximal width (µm) for each nematode from the top 0 to 5 cm, to estimate individual biomass using Andrassy's formula (Andrassy 1956) for body wet weight (WW), adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm^{-3}; µg $WW = L \times W^2/1\ 500\ 000$)[3]. C weight was calculated as 12.4 % of wet weight (Jensen 1984).

[3] The formula of Andrassy (1956) was developed on the basis of terrestrial nematodes which have a lower specific gravity than marine nematodes (i.e. 1.084 g cm^{-3} vs. 1.13 g cm^{-3}, respectively) (see Wieser 1960). Consequently, individual wet weight calculated using the formula adjusted for marine nematodes is slightly higher (6.6 %) than that calculated with Andrassy's original formula.

2.5 Stable isotope analyses

Nematodes from the top 6 cm from each core were hand-picked for $\delta^{13}C$ and $\delta^{15}N$ analysis. *Desmodora* (n = 50) and *Sabatieria* (n = 50) were picked separately, and the remaining genera were pooled to determine the "Mix" isotope value (n = 100). When not sufficiently abundant, *Desmodora* and/or *Sabatieria* were included in the "Mix" sample. Nematodes were rinsed with 2 µm filtered Milli-Q water, and then transferred to Milli-Q water in pre-combusted (550 °C, 3 h) silver cups. After elutriation, nematodes were dried overnight at 60 °C. Subsequently, we acidified samples and blanks in a desiccator containing 5 % HCl. Isotope signatures were measured on an EA-IRMS, a Flash EA 1112 coupled to a DeltaV advantage IRMS (Thermo Electron Instruments) with single low volume oxidation/reduction reactor (Carman and Fry 2002). Samples were calibrated against VPDB and N_2-Air with standards USGS40 and USGS41 (Qi et al. 2003) and all measurements were corrected for blanks. Isotope values were expressed in δ notation with respect to VPDB ($\delta^{13}C$) and air ($\delta^{15}N$): δX (‰) = $[(R_{sample}/R_{standard})-1] \times 10^3$, where X is ^{13}C or ^{15}N and R is the isotope ratio (Post 2002).

2.6 Transmission electron microscopy (TEM) and scanning transmission electron microscopy energy dispersive x-ray (STEM-EDX) analysis

Sabatieria and *Desmodora*, from the seep sediment core, were imaged with TEM to check for symbionts or visible S detoxification structures. Subsequently, we conducted STEM-EDX analysis to determine the chemical composition of internal structures. Nematodes were handled following Van Gaever et al. (2009b).

2.7 Data analysis

Individual nematode size measurements (length, width, length/width and biomass) were compared between cores (without distinguishing between sediment depth layers) using Kruskal-Wallis tests, followed by nonparametric pairwise comparisons using Behrens-Fisher tests with the R package npmc (Munzel and Hothorn 2001, Helms and Munzel 2008). In addition, we plotted nematode size measurements averaged per core and sediment depth layer as geometric means, corrected for data skewness (Middelburg et al. 1997, Soetaert et al. 2009) to examine vertical profiles. We performed multi-dimensional scaling (MDS) analysis on standardized nematode genus abundances to compare genus composition between cores. Diversity indices were ln ($\log_e$)-transformed to highlight differences. We examined feeding preferences based on: (1) $\delta^{13}C$ and $\delta^{15}N$ of *Desmodora*, *Sabatieria* and "Mix"; and (2) buccal

morphology of all genera following the classification of Wieser (1953), which assigns genera to 1 of 4 feeding types - selective deposit feeder (1A), non-selective deposit feeder (1B), epistrate feeder (2A) and predator/scavenger (2B). Isotope signatures were compared between *Sabatieria*, *Desmodora* and "Mix" using 1-way ANOVA, followed by post-hoc Tukey honest significant difference tests. Trophic diversity was computed as the reciprocal of the trophic index (Heip et al. 1985). Spearman rank correlations were computed between distance from the seep site and (1) genus diversity indices, (2) trophic diversity, and (3) stable isotope signatures. We performed univariate statistical analyses using R (R core Team 2010), and multivariate analyses and computation of diversity indices in Primer v6 (Clarke and Gorley 2006).

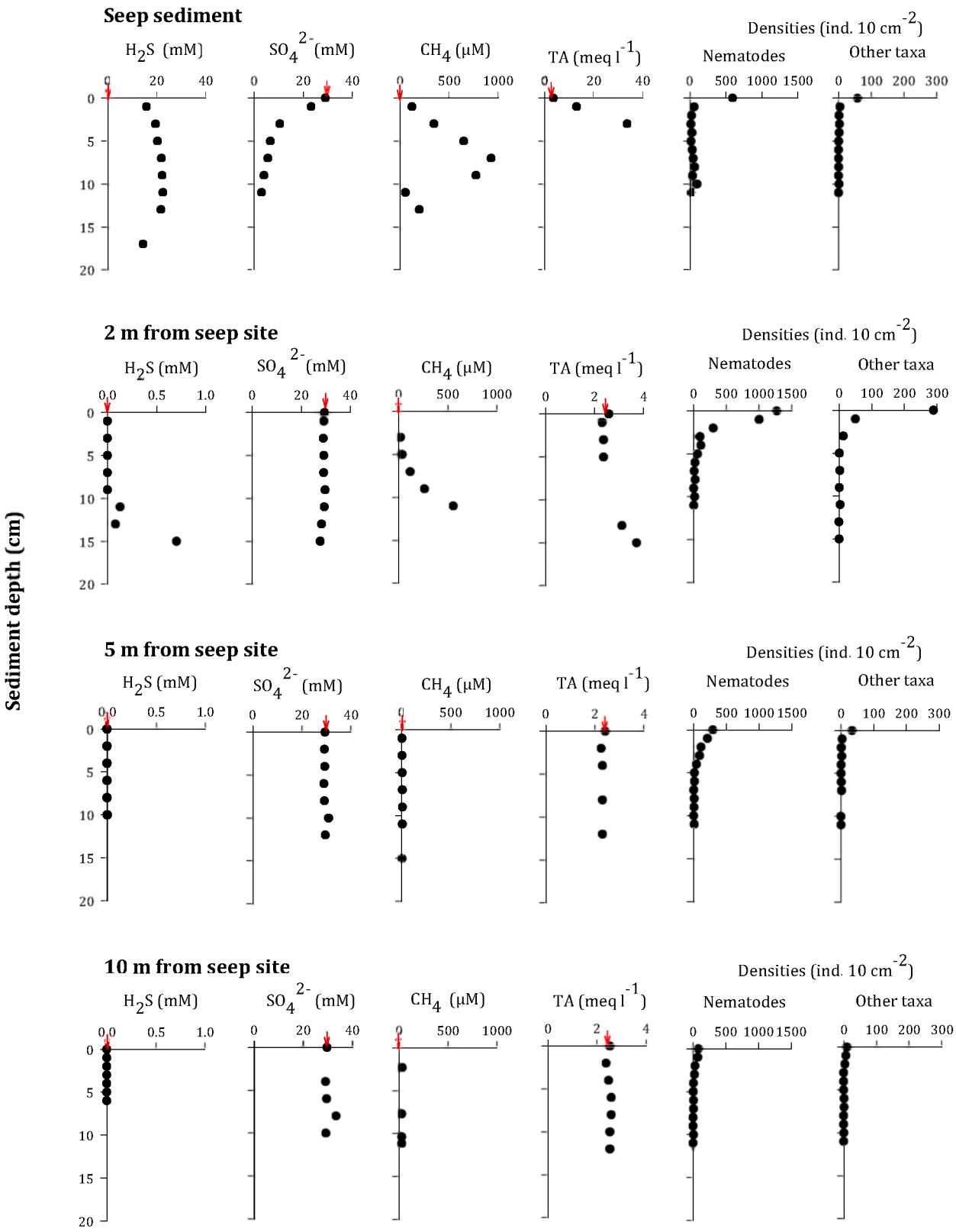

Fig. 2. *Vertical pore-water profiles of H_2S, SO_4^{2-}, CH_4 and total alkalinity (TA), and densities of nematodes and other meiofaunal taxa in relation to distance from the Darwin mud volcano seep site. Vertical red arrows on x-axes indicate seawater values. Note the different scales on the x-axes.*

3 Results

3.1 Pore-water geochemistry

Fig. 2 shows concentration-depth profiles for H_2S, SO_4^{2-}, CH_4 and TA in pore-water from all cores. From the 4 cores, only the seep sediment core showed a clear decrease in SO_4^{2-} with depth, accompanied by a peak in H_2S (up to 22 mM) and an increase in CH_4 and TA as high as 1000 µM and 33.7 meq l^{-1}, respectively. We observed very little change in SO_4^{2-} in the core taken 2 m from the seep site. However, H_2S, CH_4 and TA were enriched in the deeper sediment layers, relative to seawater. Concentrations of all of these species in cores collected 5 and 10 m from the seep site were similar to seawater concentrations and varied little with depth.

Table 2. *Meiofaunal densities (ind. 10 cm^{-2}) in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment).*

	Distance from seep site (m)					
Taxon	0	2	5	10	100	1100
Amphipoda		0.8				
Bivalvia	2.0		1.2		0.1	
Cladocera				0.4		
Cnidaria		4.3		0.8		0.3
Copepoda						
adults	7.84	121.9	12.5	3.9	16.9	7.7
nauplii	9.8	154.8	9.4	4.7	11.1	2.5
Cumacea				0.4	0.3	0.1
Gastrotricha	0.4		1.6			
Halacaroidea	4.7	2.7	0.4			
Holothuroidea		1.2			7.7	0.4
Hydrozoa	0.4					
Isopoda		3.9	0.4	0.4	0.9	0.1
Kinorhyncha	0.8			0.4		
Nematoda	725.0	2860.1	779.5	227.7	387.9	405.4
Oligochaeta	0.8	9.8	5.1			0.1
Ostracoda		0.8	1.6		1.1	0.4
Polychaeta	42.7	54.5	7.1	9.8	8.7	5.9
Tanaidacea		3.5	0.8	0.4		
Tardigrada		8.6	5.9		1.5	1.2
Total	*794.4*	*3226.9*	*825.3*	*248.4*	*436.1*	*424.3*

3.2 Meiofaunal community structure

Meiofaunal densities were highest in the core taken 2 m from the seep site (3228.8 ind. 10 cm^{-2}). Although densities in the seep sediment core (794.8 ind. 10 cm^{-2}) and in the core taken at 5 m distance (825.3 ind. 10 cm^{-2}) were considerably lower, they were still elevated compared to those collected 10 (227.7 ind. 10 cm^{-2}), 100 (436.1 ind. 10 cm^{-2}) and 1100 m (424.3 ind. 10 cm^{-2}) from the seep site (Fig. 3). Nematodes were the most abundant taxon (88.7 – 94.5 %) in all cores (Table 2). Meiofaunal densities in the seep sediment core below 1 cm decreased sharply (Fig. 2). In comparison, densities in the core taken 2 m from the seep site decreased more gradually with depth. Meiofauna in the 2 cores retrieved farthest away from seep influence penetrated deepest in the sediment.

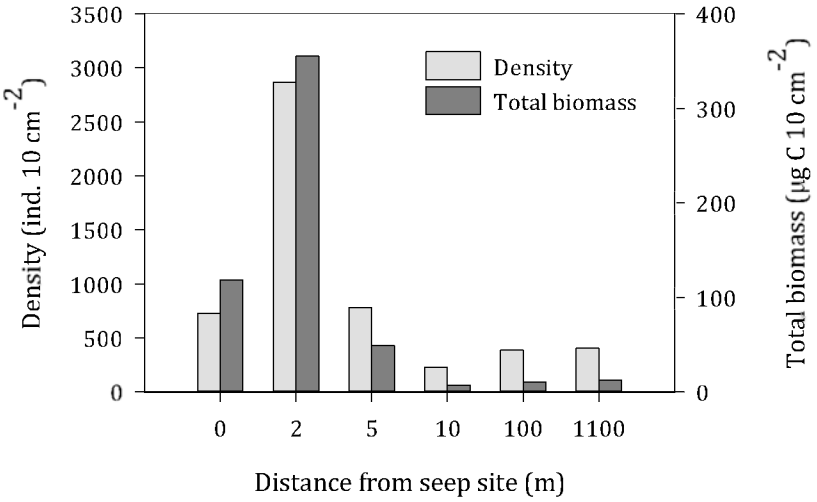

Fig. 3. *Total nematode densities and biomass in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment).*

3.3 Nematode size

Overall, total nematode biomass in the top 5 cm of the seep sediment core was ~10 × higher than that in the core taken 1100 m away (Fig. 3). Individual nematode size measurements (i.e. length, width, and biomass) differed significantly among cores ($P < 0.001$) and peaked in the seep sediment core. Also nematode length/width ratios varied significantly among cores ($P < 0.001$), with lowest ratios in the seep sediment core. *Sabatieria vasicola* and *S. punctata*, which dominated the seep sediment core, were 2416.5 ± 396.5 (mean ± SD; n = 42) and 1130.8 ± 463.1 (n = 33) µm long, respectively. Fig. 4 shows that the difference in nematode length, width and biomass between the seep sediment core and the other cores was most pronounced in the 0-1 cm layer, when compared to the deeper sediment depth layers.

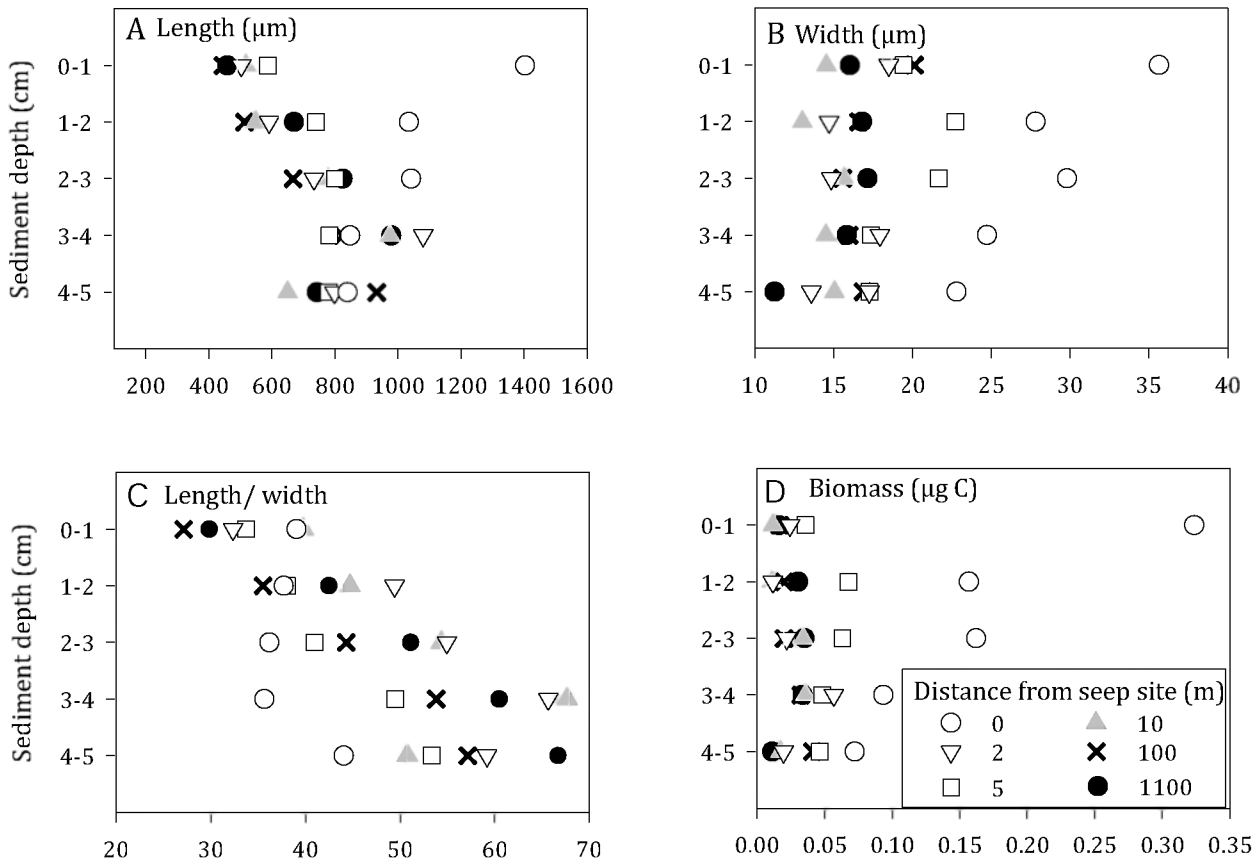

Fig. 4. *Mean nematode (A) length, (B) width, (C) length/width and (D) biomass as a function of sediment depth (cm), in relation to distance from the Darwin mud volcano seep site. 0 = seep sediment.*

3.4 Nematode community structure

Nematode genus composition varied little among cores (Fig. 5, Table 3). *Sabatieria* dominated all samples, except at 10 m from the seep site, where *Molgolaimus* (13.9 %) prevailed. All diversity indices correlated positively with distance from the seep site (Fig. 6), but these correlations were significant only for N_0 ($r = 0.89$, $P < 0.05$). *Desmodora* was only abundant (i.e. ≥ 1 % of total) within 5 m off the seep (Table 3). The *Sabatieria* species composition of all sediment cores is presented in Table 4.

Table 3. *Relative abundance (%) of the most abundant nematode genera (≥ 1 %) in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment).*

Dist:	Distance from seep site (m)											
	0		**2**		**5**		**10**		**100**		**1100**	
	Genus	%	Genus	%	Genus	%	Genus	%	Genus	%	Genus	%
	Sabatieria	56.8	*Sabatieria*	17.0	*Sabatieria*	37.6	*Molgolaimus*	13.9	*Sabatieria*	19.9	*Sabatieria*	16.3
	Desmodora	19.2	*Rhabdocoma*	14.1	*Desmodora*	6.9	*Sabatieria*	12.0	*Thalassomonhystera*	7.5	*Acantholaimus*	9.3
	Desmoscolex	5.5	*Amphimonhystrella*	11.6	*Thalassomonhystera*	6.4	*Acantholaimus*	6.0	*Molgolaimus*	6.0	*Amphimonhystrella*	7.3
	Etmolaimidae n.gen.	2.6	*Desmodora*	11.3	*Halalaimus*	5.3	*Daptonema*	5.8	*Acantholaimus*	5.6	*Theristus*	7.2
	Prototricoma	2.1	*Tricoma*	8.2	*Acantholaimus*	4.8	*Thalassomonhystera*	5.1	*Halalaimus*	3.8	*Thalassomonhystera*	7.1
	Tricoma	1.8	*Daptonema*	4.5	*Molgolaimus*	4.3	*Halalaimus*	5.0	*Diplopeltula*	3.4	*Molgolaimus*	4.8
	Linhomoeus	1.6	*Molgolaimus*	3.5	*Amphimonhystrella*	2.8	*Amphimonhystrella*	4.3	*Amphimonhystrella*	3.2	*Halalaimus*	3.2
	Comesa	1.5	*Ethmolaimidae n.gen.*	3.4	*Microlaimus*	2.1	*Theristus*	4.1	*Greefiella*	3.1	*Daptonema*	3.0
	Theristus	1.3	*Thalassomonhystera*	2.6	*Desmoscolex*	2.0	*Sphaerolaimus*	3.6	*Hopperia*	3.0	*Neochromadora*	3.0
	Aegialoalaimus	1.3	*Wieseria*	2.0	*Antarcticonema*	1.6	*Nemanema*	3.5	*Theristus*	2.6	*Leptolaimus*	2.8
	Parasphaerolaimus	1.3	*Leptolaimoides*	2.0	*Aegialoalaimus*	1.6	*Syringolaimus*	2.6	*Neochromadora*	2.5	*Chromadorina*	2.2
			Desmoscolex	2.0	*Neochromadora*	1.5	*Leptolaimus*	2.5	*Leptolaimus*	2.4	*Diplopeltula*	2.1
			Microlaimus	1.8	*Parasphaerolaimus*	1.3	*Neochromadora*	2.3	*Cervonema*	2.2	*Hopperia*	1.9
			Neochromadora	1.5	*Tricoma*	1.2	*Oxystomina*	2.2	*Prototricoma*	2.1	*Omicronema*	1.8
			Vasostoma	1.4	*Chromadorita*	1.2	*Microlaimus*	1.9	*Desmoscolex*	2.1	*Microlaimus*	1.7
			Neotonchus	1.1	*Leptolaimus*	1.1	*Aegialoalaimus*	1.7	*Omicronema*	1.8	*Desmoscolex*	1.7
			Aegialoalaimus	1.0	*Nyctonema*	1.1	*Linhystera*	1.7	*Daptonema*	1.6	*Cervonema*	1.7
			Leptolaimus	1.0	*Metasphaerolaimus*	1.0	*Halichoanolaimus*	1.6	*Tricoma*	1.6	*Tricoma*	1.4
					Hopperia	1.0	*Metadesmolaimus*	1.4	*Desmotricoma*	1.5	*Antarcticonema*	1.3
							Rhabdocoma	1.2	*Halichoanolaimus*	1.4	*Doliolaimus*	1.3
							Leptolaimoides	1.1	*Monhystrella*	1.4	*Syringolaimus*	1.2
							Prototricoma	1.1	*Microlaimus*	1.3	*Oxystomina*	1.2
									Campylaimus	1.2	*Aegialoalaimus*	1.0
									Oxystomina	1.1	*Comesa*	1.0
									Aegialoalaimus	1.1	*Sphaerolaimus*	1.0

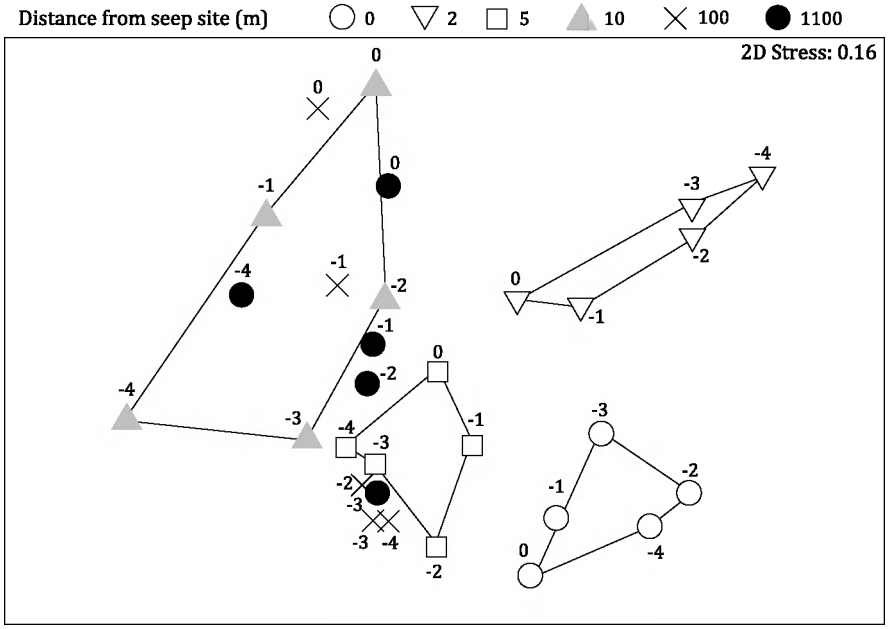

Fig. 5. *Multi-dimensional scaling plot of standardized genus abundance data in relation to distance from the Darwin mud volcano seep site. Numbers = sediment depth (cm). 0 = seep sediment. Contour plots for the cores collected at 100 and 1100 m from the seep site overlap and are not shown.*

3.5 Nematode feeding ecology

$\delta^{13}C$ ranged between -40.7 and -21.3 ‰, and $\delta^{15}N$ between 0.9 and 15.3 ‰ (Fig. 7). $\delta^{13}C$ ($r = 0.34$, $P = 0.14$) and $\delta^{15}N$ ($r = 0.24$, $P = 0.28$) became heavier with increasing distance from the seep site, though the correlations were not significant. No clear pattern emerged when plotting stable isotope signatures (mean ± SD) versus sediment depth (Fig. 7). "Mix" ($\delta^{13}C$: - 31.2 ± 4.9 ‰, $\delta^{15}N$: 7.11 ± 3.9 ‰) was significantly more enriched in ^{13}C ($P < 0.05$) and ^{15}N ($P < 0.02$) than *Desmodora* ($\delta^{13}C$: -38.5 ± 2.0 ‰, $\delta^{15}N$: 4.6 ± 2.2 ‰). *Sabatieria* ($\delta^{13}C$: -36.3 ± 2.4 ‰, $\delta^{15}N$: 6.9 ± 1.5 ‰) and *Desmodora* displayed similar isotope values ($\delta^{13}C$: $P = 0.67$, $\delta^{15}N$: $P = 0.43$). Based on buccal morphology, deposit feeders (1A + 1B) dominated all cores (data not shown), although trophic diversity increased with increasing distance from the seep site ($r = 0.83$, $P = 0.06$) and levelled off at 10 m distance (Fig. 8).

Table 4. *Relative abundance of* Sabatieria *species (%) in the top 5 cm of the sediment cores in relation to distance (Dist.; m) from the Darwin mud volcano seep site (0 = seep sediment).*

Dist.:	**0**		**2**		**5**		**10**		**100**		**1100**	
	Species	%	Species	%	Species	%	Species	%	Species	%	Species	%
	S. vasicola	44.6	*S. bitumen*	66.8	*S. bitumen*	39.7	*S. bitumen*	56.6	*S. stekhoveni*	30.4	*S. stekhoveni*	35.1
	S. punctata	29.5	*S. propisinna*	21.5	*S. demani*	17.2	*S. demani*	16.9	*S. ornata*	21.8	*S. propisinna*	24.5
	S. stekhoveni	17.9	*S. ornata*	10.2	*S. propisinna*	17.2	*S. propisinna*	12.3	*S. aff. breviseta*	16.9	*S. aff. breviseta*	15.0
	S. aff. breviseta	4.8	*S. stekhoveni*	1.6	*S. aff. breviseta*	15.0	*S. stekhoveni*	10.4	*S. bitumen*	15.0	*S. conicauda*	8.5
	S. ornata	2.2			*S. stekhoveni*	9.2	*S. ornata*	3.9	*S. demani*	9.4	*S. punctata*	6.4
	S. propisinna	0.5			*S. ornata*	1.7			*S. conicauda*	4.8	*S. lawsi*	5.9
	S. demani	0.3							*S. propisinna*	1.7	*S. demani*	3.1
	S. conicauda	0.2									*S. ornata*	1.0
											S. vasicola	0.5

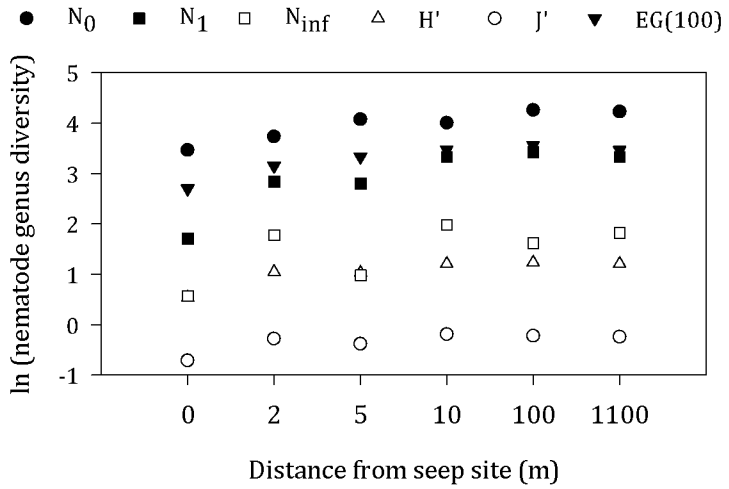

Fig. 6. *ln-transformed diversity indices based on abundances of nematode genera in relation to distance from the Darwin mud volcano seep site. N_0, N_1, N_{inf}: Hill's numbers; H': Shannon-Wiener diversity index; J': Pielou's evenness number; EG(100): expected number of genera for n = 100. 0 = seep sediment.*

3.6 TEM and STEM-EDX

Some TEM sections showed intact, but few (maximum 5) bacterial cells bordering the cuticle of *Desmodora* (Fig. 9). Additionally, electron-lucent structures were observed near the cuticle (Fig. 9B). STEM-EDX analysis showed these contained trace amounts of S. In *Sabatieria*, no symbionts or detoxification structures were observed.

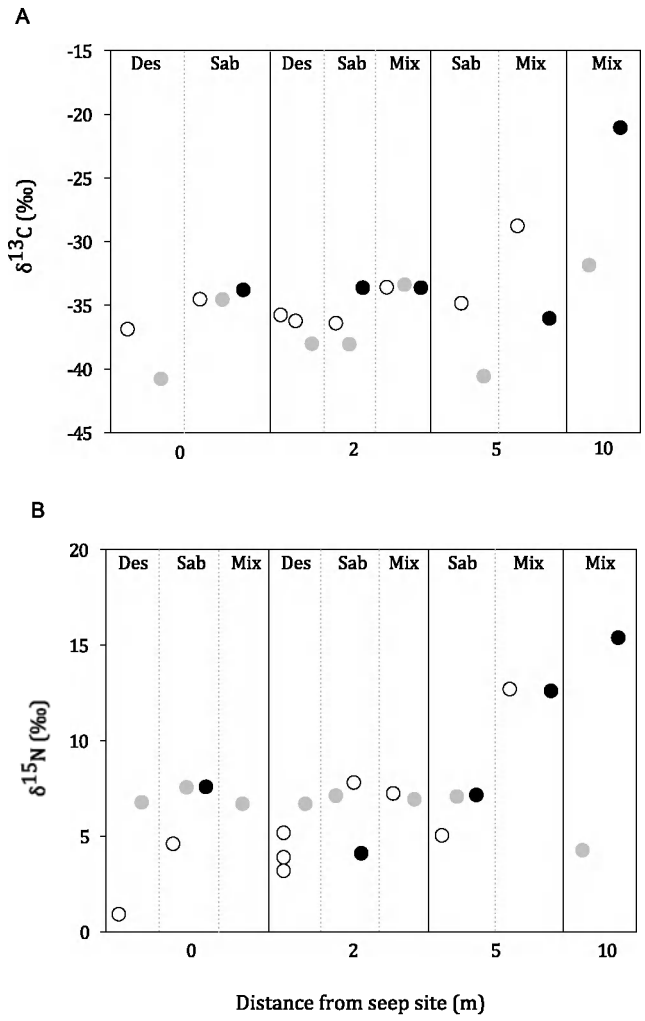

Fig. 7. Desmodora *(Des)*, Sabatieria *(Sab) and "Mix" (A) carbon and (B) nitrogen isotope signatures in relation to distance from the Darwin mud volcano seep site. 0 = seep sediment. At 10 m distance, no $\delta^{13}C$ was available for 0 to 2 cm owing to the low amount of C, making the isotope value unreliable. Sediment layers: white symbols = 0 to 2 cm; grey symbols = 2 to 4 cm; black symbols = 4 to 6 cm.*

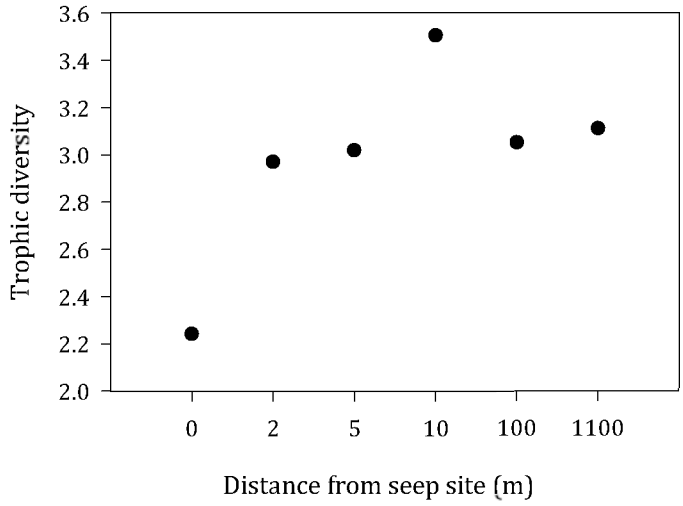

Fig. 8. *Nematode trophic diversity in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment).*

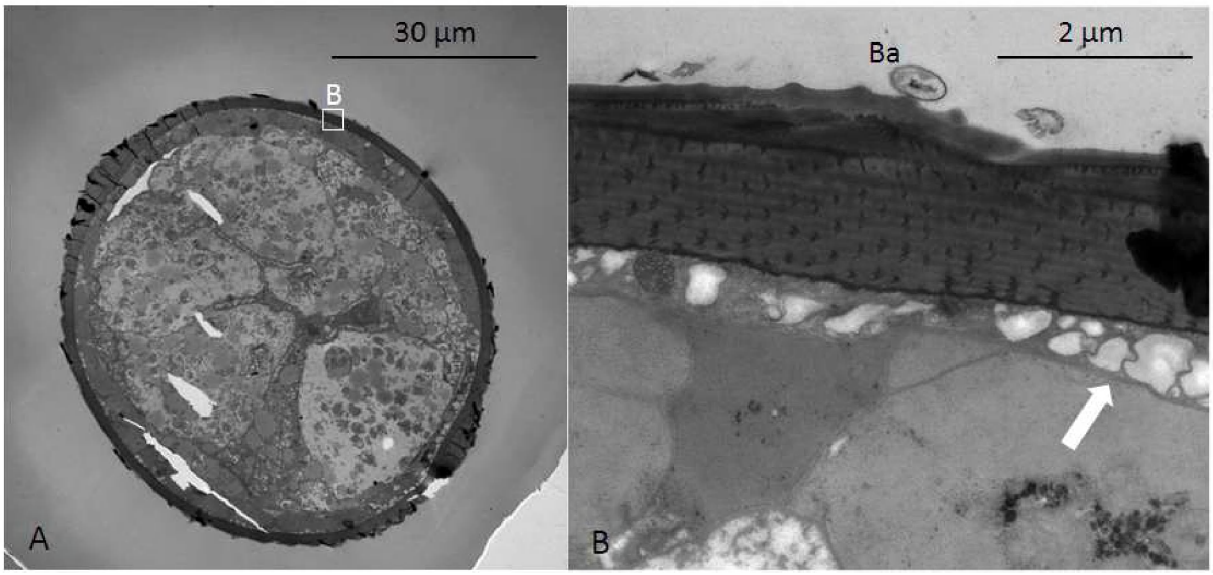

Fig. 9. *Transmission electron microscope micrograph of a cross-section of* Desmodora *from the Darwin mud volcano seep sediment core: (A) overview and (B) detailed depiction showing bacteria associated with the cuticle. The white arrow points to electron-lucent structures, possibly containing sulphur prior to ethanol dehydration (see 4.2). Ba: bacterial cell.*

4 Discussion

4.1 CH_4 seepage and spatial variability in pore-water geochemistry

Elevated pore-water CH_4 levels in the Darwin MV seep sediment core indicated a CH_4 flux from below the sediment surface, corroborating the gas escape from the seep sediments during sampling. However, CH_4 concentrations dropped from 1 mM down to < 0.001 mM over a 10 m distance, suggesting focused flow. Pore-fluid analyses indicated some anaerobic consumption by microbes (i.e. anaerobic oxidation of methane or AOM): SO_4^{2-} decreased rapidly with depth in the seep-site pore-fluids, accompanied by an increase in TA and elevated H_2S concentrations (Reeburgh 1976, Boetius et al. 2000, Knittel and Boetius 2009). The relatively small enrichments in H_2S, CH_4 and TA in the core taken 2 m from the seep site suggest AOM presence here as well, but likely concentrated at depths exceeding the core length. The constancy in the concentrations of SO_4^{2-}, TA, H_2S and CH_4 with depth in the cores taken at 5 and 10 m distance suggests an absence of AOM. The high spatial variability in CH_4 flow at the Darwin MV illustrates the difficulty in taking replicate samples for pore-water geochemistry and associated fauna at seeps.

4.2 Influence of pore-water geochemistry on meiofaunal distribution and tolerance to high H_2S levels

At the Darwin MV, meiofaunal densities were much higher in and immediately near the seep site (2 m) compared to at the sites showing no sign of seep influence in terms of pore-water geochemistry. Accordingly, Vanreusel et al. (2010a) showed elevated meiofaunal standing stock in seep compared to non-seep sediments for several other systems. The seep sediment core had high H_2S content (up to 22 mM), as shown for several other seeps (Barry et al. 1997, Sahling et al. 2002, Levin et al. 2003). These high H_2S levels affected the vertical distribution in the sediment, in that the proportion of meiofauna confined to the sediment surface was highest in this core. Tolerance of high H_2S levels was genus (and species) specific. *Sabatieria* and *Desmodora*, which dominated the seep sediment core, were more tolerant to high H_2S than genera absent from this core.

In sulphidic environments, bacterial symbionts can help to detoxify H_2S (Ott et al. 2004). In bathyal oxygen minimum zone sediments, *Desmodora masira* had ectosymbionts (Bernhard et al. 2000). Although in our study, TEM cross-sections paralleled the annuli (in contrast to Bernhard et al. 2000), the few intact bacteria observed in only some of the sections indicate that *Desmodora* from the seep sediment core did not harbour ectosymbionts. TEM showed electron-lucent structures near the cuticle, resembling the sulphur inclusions described by Thiermann et

al. (2000). STEM-EDX analysis only detected trace amounts of S. However, elemental sulphur is known to leach out of vesicles during the chemical fixation, dehydration and resin infiltration of biological samples, which may explain the low sulphur content (Lechaire et al. 2006). Both Soetaert and al. (2002) and Schratzberger et al. (2004) hypothesized that longer species are more mobile relative to shorter ones. Increased body length may represent an adaptation to unfavourable (anoxic or sulphidic) conditions, allowing for a faster migration between anoxic, sulphidic and oxic, H_2S-free sediments (Levin et al. 2005). Accordingly, *S. punctata*, and *S. vasicola*, which dominated the seep sediment core, were amongst the longest nematodes in the present study.

4.3 Meiofaunal and nematode community structure

Meiofaunal density patterns were mainly driven by the dominant taxon, i.e. the nematodes. Nematodes prevail in most seep habitats (Shirayama and Ohta 1990, Robinson et al. 2004, Van Gaever et al. 2009a, c), although some habitats are dominated by copepods (Van Gaever et al. 2006). In the Darwin MV seep sediment core, meiofauna-sized polychaetes were subdominant, similar to the mussel beds at the REGAB seep in the Gulf of Guinea (Van Gaever et al. 2009a).

Nematode genus composition clearly differed between cores with and without CH_4 flow. Thus, CH_4 flow affected not only densities and biomass, but also composition. Genus diversity was lowest in the seep sediment core and increased in cores farther from the seep site, as shown in previous studies (Van Gaever et al. 2009a, c). *Desmodora* and *Sabatieria* also dominated the REGAB seep in the Gulf of Guinea (Van Gaever et al. 2009a), although in association with different habitats: the REGAB samples originated from clam and mussel fields. *S. vasicola* and *S. punctata*, which dominated the Darwin MV seep sediment core, also occur in shallow waters (Vitiello 1970, Jensen et al. 1992, Franco et al. 2008). Accordingly, the dominant species at the REGAB seep (*S. mortenseni*), the Arctic Håkon Mosby MV (*Halomonhystera disjuncta*) (Van Gaever et al. 2006) and the Nordic Nyegga seep (*Terschellingia longicaudata*) (Van Gaever et al. 2009c) inhabit shallow waters as well. Molecular analysis confirmed that *H. disjuncta* dominating the Håkon Mosby MV bacterial mats was the same species as those found in the intertidal North Sea, though it may represent (at least) one new cryptic species within the *H. disjuncta* species complex (Van Gaever et al. 2009c). The presence of these species in both shallow waters and at a deep-sea seep suggests a possible connection between these habitats, rather than between deep-sea seeps (Van Gaever et al. 2009c).

4.4 Nematode feeding ecology

$\delta^{13}C$ values suggest thiotrophic C is part of the nematode diet up to 10 m from the seep site. Except for "Mix" in the 4 to 6 cm sediment layer, which displayed a $\delta^{13}C$ of -21.3 ‰, all $\delta^{13}C$ were less than -28 ‰. Organic matter produced through sulphur-oxidation has an average $\delta^{13}C$ of -30 ‰ (RubisCO I) or -11 ‰ (RubisCO II), depending on the Rubisco enzyme involved (Robinson and Cavanaugh 1995). In comparison, photosynthetic C is characterized by a $\delta^{13}C$ between -18 and -28 ‰ (Stewart et al. 2005), and CH_4-derived C is more depleted in ^{13}C ($\delta^{13}C$ < -50 ‰) (Levin and Michener 2002). Since we did not sample potential C sources for stable isotope analysis, we cannot estimate their relative contribution to the nematode diet. Nonetheless, a decrease in thiotrophic (RubisCO I) and an increase in photosynthetic C in the nematode diet farther from the seep site are implied by (1) the thicker hemipelagic sediment veneer on top of the cores, suggesting a higher availability of photosynthetic C, (2) an increase in trophic diversity, and (3) heavier $\delta^{13}C$.

Spies and DesMarias (1983) and Van Gaever et al. (2006, 2009b) reported direct nematode consumption of sulphur-oxidizers. These bacteria live at the interface between oxic and anoxic sediments, where H_2S levels are ≤ 1 µM (Robertson and Kuenen 2006, Preisler et al. 2007). We doubt these bacteria inhabited the seep sediments given the absence of bacterial mats and the high H_2S levels at greater depth. In the core collected 2 m from the seep site, we observed no net SO_4^{2-} production, expected in the presence of sulphur-oxidisers. Nematodes can indirectly consume sulphur-oxidizers by assimilating dissolved organic matter (DOM) released upon bacterial lysis. Jensen (1987a) suggested thiobiotic nematodes feed, at least partially, on DOM. However, further evidence is needed to support this hypothesis. As with other seeps (Vanreusel et al. 2010a), deposit feeders dominated. Finally, although this exploratory study hints at how meiofauna interact with the seep environment, much more, high-resolution research is required to understand their tolerance of sulphide, trophic interactions and dispersal capacities.

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Dave McCarthy

Chapter 3

Nematode feeding strategies and the fate of dissolved organic matter carbon in different deep-sea sedimentary environments

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ABSTRACT – Sediments sampled from the Galicia Bank seamount and the adjacent slope (northeast Atlantic), and from the western Mediterranean were injected onboard with ^{13}C-enriched dissolved organic matter (DOM) to evaluate nematode feeding strategies and the fate of DOM carbon in different benthic environments.

We hypothesized that nematode ^{13}C label assimilation resulted from either direct DOM ingestion or feeding on ^{13}C labeled bacteria. The time-series (1, 7 and 14 days) experiment at the seamount station was the first study to reveal a higher ^{13}C enrichment of nematodes than bacteria and sediments after 7 days. Although isotope dynamics indicated that both DOM and bacteria were plausible candidate food sources, the contribution to nematode secondary production and metabolic requirements (estimated from biomass-dependent respiration rates) was higher for bacteria than for DOM at all stations. The seamount nematode community showed higher carbon assimilation rates than the northeast Atlantic and Mediterranean assemblages, which may reflect an adaptation to the food-poor environment. Our results suggested that the trophic importance of bacteria did not depend on the amount of labile sedimentary organic matter. Furthermore, there was a discrepancy between carbon assimilation rates observed in the experiments and the feeding type classification, based on buccal morphology. Stations with a similar feeding type composition (northeast Atlantic stations) showed large differences in uptake, whilst the nematode assemblages at the two slope stations, which had a differing trophic structure, took up similar amounts of the DOM associated carbon.

Slope sediments were injected with glucose ("simple" DOM) or "complex" diatom-derived DOM to investigate the influence of the composition of DOM on carbon assimilation. Our results did not indicate substantial differences in carbon processing related to the complexity of the DOM substrate. The quantity of processed carbon (5–42 % of added DOM) was determined by total consumer biomass (dominated by bacteria), and was primarily respired. The bulk of the added ^{13}C-DOM was not ingested by the benthic biota under study, and a considerable fraction was possibly adsorbed to the sediment grains.

1 Introduction

Nematodes generally represent the bulk of metazoan biomass and abundance in deep-sea sediments (Rex et al. 2006, Wei et al. 2010). In coastal food webs, these organisms are believed to be important because of their high metabolic and reproductive rates and intermediate trophic position (between bacteria/detritus/microalgae and macrofauna/megafauna), in conjunction with their ubiquity and high standing stock (Giere 2009). Whether this also applies to deep-sea species is unknown, since information on their life history strategies is entirely lacking. Feeding strategies of nematodes are generally inferred from the morphology of their buccal cavity (Wieser 1953). However, a disadvantage of Wieser's trophic classification scheme is that it does not take into account feeding selectivity nor flexibility (Olafsson et al. 1999, Moens et al. 2004). Moreover, empirical verification of the classification is limited to a few observational studies on shallow-water nematodes (Jensen 1987a, Moens and Vincx 1997), whilst the applicability of the scheme for deep-sea species is not proven.

According to Wieser's classification, the dominant nematode genera in the deep sea are deposit (feeding types 1A + 1B; e.g. *Monhystrella* and *Halalaimus*) or epistrate (2A; e.g. *Acantholaimus*) feeders, which can be regarded as potential bacterivores (Tietjen 1984, Jensen 1988, Soetaert and Heip 1995). In shallow-water environments, deposit feeding nematodes feed largely on bacteria and protozoans, while epistrate feeders feed on diatoms (Moens and Vincx 1997). Nonetheless, epistrate feeding nematodes are often abundant in deep-sea sediments despite the lack of fresh and intact diatom cells, so it seems plausible that in this environment, epistrate feeders use their small teeth to scrape off microbial coatings from sediment particles or mucus threads (Moens and Vincx 1997). The quantitative significance of bacteria to the diet of predatory/scavenging nematodes (feeding type 2B) is considered negligible, since (1) Moens et al. (1999c) detected no to very limited uptake of bacterial cells by intertidal predatory nematodes, (2) Fonseca and Gallucci (2008) found that the gut content of deep-sea predatory

nematodes often contained nematode remains and (3) during nematode identification, we observed several predatory specimens in the process of swallowing another nematode.

Isotope tracer experiments are a straightforward tool for elucidating carbon flows in deep-sea benthic food webs (Moodley et al. 2002, 2005, Moens et al. 2007, Gontikaki et al. 2011a). Because the natural abundance of the heavy ^{13}C isotope is negligible compared to that of the light ^{12}C isotope (1 vs. 99 % of total abundance, respectively) (Sulzman 2007), the fate of a particular carbon compound can be easily followed when it has an elevated ^{13}C content. Upon assimilation of a ^{13}C-enriched food source, part of the ^{13}C-tracer is incorporated in consumer biomass, and consequently, uptake can be quantified by assessing the increase in the ^{13}C:^{12}C ratio of consumers after isotope tracer addition. Deep-sea nematodes have been subjected to isotope tracer experiments using lab-cultured, freeze-dried ^{13}C-enriched algae (Moodley et al. 2002, Witte et al. 2003b, Ingels et al. 2010, 2011a, Gontikaki et al. 2011a), bacteria (Ingels et al. 2010, 2011a), or cyanobacteria (Moens et al. 2007). More recently, Guilini et al. (2010) injected Arctic deep-sea sediments with ^{13}C labeled dissolved organic matter to enrich the autochthonous heterotrophic bacteria to examine bacterivory by the nematodes. These feeding experiments showed very limited assimilation of the added label, suggesting that nematodes do not rely heavily on any of these food sources.

The lack of bacterial consumption in the feeding experiments by Ingels et al. (2010, 2011a) and Guilini et al. (2010) does not corroborate with the prevalence of potential bacterial feeders inferred from buccal morphology. Moreover, several studies found significant positive correlations between nematode and bacterial standing stocks in the deep sea, which may point to a trophic link (Rowe et al. 1991, Vanreusel et al. 1995b, Hoste et al. 2007). The negligible bacterial uptake observed by Ingels et al. (2010, 2011a) may be related to the origin (i.e. lab-cultured, shallow-water bacteria) and/or state of the bacteria (i.e. freeze-dried) offered to the nematode community. For instance, Cnudde et al. (2011) demonstrated that copepods preferred fresh over freeze-dried diatoms in an experimental setting. Furthermore, Ingels et al. (2010, 2011a) placed the labeled, freeze-dried bacteria at the sediment surface which is not necessarily where (most) nematodes engage in feeding activities.

The injection of stable isotope labeled dissolved organic matter (DOM) into marine sediments allows the quantification of carbon flows through nematodes and bacteria (Boschker and Middelburg 2002, van Oevelen et al. 2006a, Guilini et al. 2010). Benthic heterotrophic bacteria assimilate label by consuming DOM directly (Carlson 2002), whereas nematodes can incorporate ^{13}C-DOM starting from two potential end-members: the DOM itself (direct DOM ingestion was demonstrated for shallow-water nematodes by Chia and Warwick 1969,

Montagna 1984, Riemann et al. 1990) or labeled bacteria. Time-series experiments elucidate the temporal dynamics of the isotope label, which can help distinguish between these two uptake pathways (van Oevelen et al. 2006b, Guilini et al. 2010). Important benefits of this experimental approach are (1) the labelling of the natural, *in situ* benthic bacterial community (opposed to the addition of freeze-dried, "alien" bacteria) and (2) the occurrence of enriched bacteria throughout the sediment column (vs. enriched bacteria only present at the sediment-water interface).

We conducted an on-board time-series experiment (1, 7 and 14 days) with ^{13}C- enriched glucose on sediments from the Galicia Bank (GB) seamount (1200 m, northeast Atlantic) to identify nematode feeding strategies. Sediment cores were sliced horizontally to assess sediment depth-dependent feeding behaviour. In addition, we conducted experiments with sediments from two 1900 m deep slope stations (from the northeast Atlantic and the western Mediterranean), for which we quantified isotope enrichment at day 7 only and provided either ^{13}C-glucose as a "simple" DOM substrate or "complex" DOM extracted from ^{13}C-enriched diatoms (hereafter referred to as diatom DOM). The difference in geomorphologic background between the 1200 (seamount) and 1900 m (slope) stations signified a contrast in hydrodynamic regime and sediment texture. The summit of the GB experiences strong intertidal currents (median current speed: 8 cm s^{-1}, up to 30 cm s^{-1}; Duineveld et al. 2004) winnowing the fine sediments and leaving behind a thick cover of foraminiferal ooze with high median grain size (Flach et al. 2002, van Weering et al. 2002, Duineveld et al. 2004). In comparison, the northeast Atlantic (average current speed: 4.1 cm s^{-1}; R. Jeffreys, pers. obs.) and Mediterranean (average current speed: 3.8 cm s^{-1}; Jeffreys et al. 2011) slope stations were more tranquil.

The results of all experiments were used to quantify the importance of bacteria and DOM to the nematode diet under either a strictly bacterivorous or a strict DOM feeding strategy. For each hypothetical feeding strategy, we (a) compared the ^{13}C enrichment of the nematodes with that of both candidate food sources and (b) calculated the contribution of DOM and bacterial carbon uptake to nematode carbon demands inferred from respiration rates and nematode growth efficiencies. These empirical results were also compared with the findings from the feeding type classification *sensu* Wieser (1953). Besides nematode feeding strategies, the experimental data were used to quantify the potential flow of "simple" and "complex" DOM carbon through the nematodes and the bacteria. The influence of water depth (Soetaert and Heip 1995), sediment texture (Tietjen 1984, Carman et al. 1987) and hydrodynamic regime (Levin and Thomas 1989) on the biomass or generic/trophic composition of nematode assemblages has been documented in several deep-sea studies. Bacterial standing stock was shown to be impacted positively (Thistle et al. 1985) or negatively (Levin and Thomas 1989) by hydrodynamic stress. Because our stations differed in these environmental factors, we could evaluate their influence and that

of the resultant community composition on nematode feeding strategies and the fate of DOM carbon.

2 Materials and methods

2.1 Study sites and sampling strategy

We collected sediment samples in 2008 in the region of the Galicia Bank (GB) seamount (northeast Atlantic) at 1200 (RV *Belgica*) and 1900 m water depth (RV *Pelagia*), and in 2009 at 1900 m depth in the Algero-Provençal Sea, in the western Mediterranean (RV *Pelagia*) (Fig. 1, Table 1). These two regions were selected within the multidisciplinary EuroDEEP-funded research project called "BIOdiversity and Ecosystem FUNctioning in southern European deep-sea environments: from viruses to megafauna" (BIOFUN). The GB is situated on the north-western Iberian margin, which is characterized by relatively high primary productivity owing to intense, wind-driven upwelling during spring and summer (220 g C m^{-2} yr^{-1}) (McClain et al. 1986, Joint et al. 2002). In comparison, the Algero-Provençal Sea has lower surface primary productivity (~ 153 g C m^{-2} yr^{-1}; Bosc et al. 2004) and the high water temperature (13 °C) accelerates the degradation of surface-produced OM sinking to the seabed, such that the OM pool available to the Mediterranean benthos is presumed to be more refractory than that for the benthic organisms in the GB region. Station GB1200 was situated on the GB seamount, whereas GB1900 (GB region, Iberian continental slope) and WMed1900 (southeast of Menorca in the western Mediterranean) were located on the slope (Fig. 1B-C).

Table 1. *Details of the stations sampled in the Galicia Bank (GB) region and in the Western Mediterranean (WMed). Temp.: bottom-water temperature. For the GB stations, a water depth range is given over the three replicate deployments; for station WMed1900, the position of only one deployment was noted. Latitude and longitude are expressed in decimal degrees. Pore-water conc. = pore-water concentration*

		GB1200	**GB1900**	**WMed1900**
Location		GB	GB	WMed
Water depth (m)		1139-1141	1770-1896	1582
Date sampled		June 2008	Oct-Sept 2008	Nov 2009
Lat		42.9121	42.4607	39.4167
Long		-11.7522	- 10.6547	4.2667
Temp. (°C)		10	4	13
Horizontal slicing	control samples	0-1, 1-2, 2-3, 3-4 cm	0-1 cm, 1-2 cm	0-2 cm
	experimental samples	0-1, 1-2, 2-3, 3-4 cm	0-2 cm	0-2 cm
Storage	control samples	frozen	frozen	formalin
	experimental samples	frozen	frozen	formalin
^{13}C labeled DOM substrates	type	glucose	glucose and diatom DOM	glucose and diatom DOM
	amount (mg C m^{-2})	60	100	100
	pore-water conc. (µM)	135.1	568.5	568
Injection mode		syringe gradually emptied over 5 cm sediment depth	syringe entirely emptied at 1 cm sediment depth	syringe entirely emptied at 1 cm sediment depth

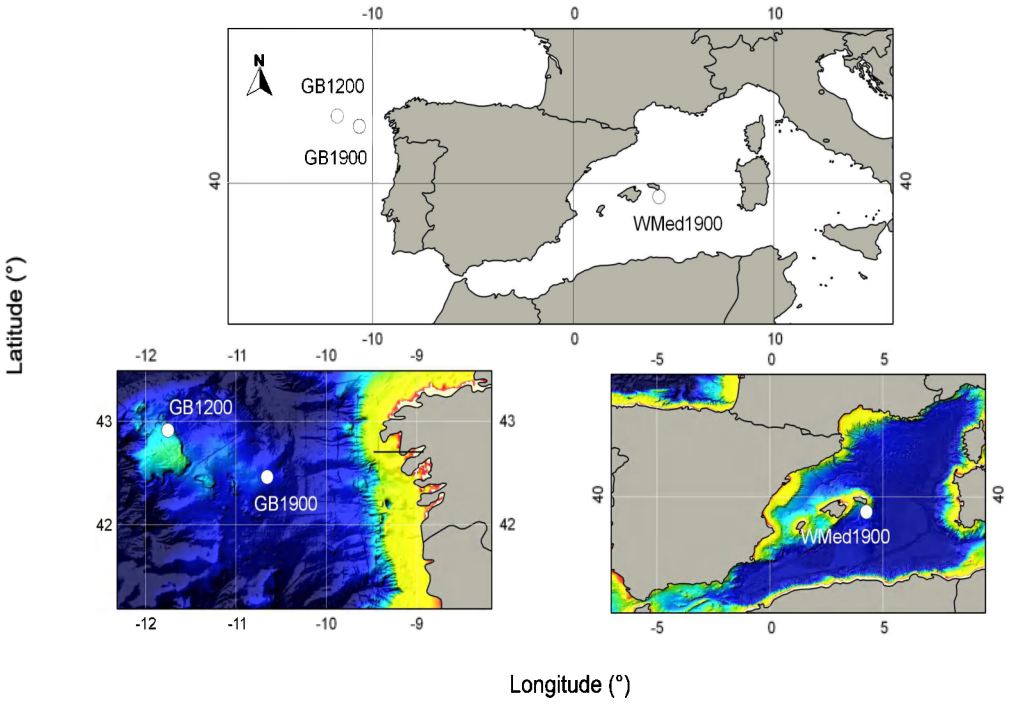

Fig.1. *(A) Overview map of stations GB1200, GB1900 and WMed1900. Bathymetry of (B) the Galicia Bank (GB) region and (C) the Western Mediterranean (WMed). GEBCO bathymetric data were downloaded from EMODnet on 16/03/2012.*

At all stations, we collected three replicate field samples for granulometric and biogeochemical analysis (total organic carbon, total nitrogen and total organic matter content) and another three replicates for nematode community analysis. In the GB region, we obtained field samples by sub-sampling boxcores with polycarbonate multicorer tubes of 9.5 (surface area: 70.88 cm^2) and 10 cm (surface area: 78.54 cm^2) internal diameter (i.d.) at the 1900 and 1200 m station, respectively. In the western Mediterranean, multicorer samples (i.d. 6 cm; surface area: 28.35 cm^2) were collected. Samples for granulometric and biogeochemical analysis were stored frozen, whereas sediment samples for nematode community analysis were fixed in seawater-buffered 4 % formalin. In the western Mediterranean, each of the three replicate samples for community analysis comprised two pooled cores amounting to a sample surface area of 56.5 cm^2. All field samples were sliced per cm down to 4 cm sediment depth.

2.2 Field samples

2.2.1 Granulometric and biogeochemical analysis

Grain-size analysis was conducted using a Malvern Mastersizer hydro 2000 G. Sediment fractions were classified according to the Wentworth scale (Wentworth 1922). The sorting coefficient (SC) of the sediment was determined following Giere (2009). After freeze-drying and homogenization, samples were acidified with dilute HCl until complete decarbonisation. Next, samples were dried and total organic carbon (TOC) and nitrogen (TN) content were measured with a Flash EA 1112+ MAs 200 elemental analyser (Thermo Interscience). The fraction of total organic matter (TOM) in the sediment was determined after combustion at 550 °C.

2.2.2 Nematode community analysis

We washed the formalin-fixed samples over a 32 µm mesh sieve and extracted the meiofauna from the sediment by Ludox centrifugation, followed by staining with Rose Bengal (Heip et al. 1985). All nematodes in each sample were counted and converted to densities per 10 cm^2. Where possible, ca. 100 nematodes were hand-picked from each sample and identified to genus level. Since it was difficult to distinguish between *Microlaimus* and *Aponema*, specimens belonging to one of these genera were allocated to a *Microlaimus/Aponema* genus complex. Nematodes were grouped into four feeding types on the basis of buccal morphology *sensu* Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate feeders (2A), and scavengers/predators (2B). In this study, feeding types 1A, 1B and 2A were considered potential bacterivores.

Because total carbon uptake is determined by consumer biomass (see 2.3.4), we assessed genus and trophic composition based on the relative biomass of genera and feeding types. Therefore, we measured length (L, µm) and maximal width (W, µm) for each nematode to estimate individual wet weight (WW) using Andrassy's (1956) formula, adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm^{-3}; µg $WW = L \times W^2/1\ 500\ 000$). Individual biomass (B) in terms of µg C ind^{-1} was then calculated as 12.4 % of WW (Jensen 1984), averaged per genus/feeding type and multiplied by relative genus/feeding type densities to obtain genus/feeding type biomass relative to total nematode biomass (i.e. "relative genus/feeding type biomass"). Relative genus and feeding type densities in the 0-2 cm sediment layer for all three stations were obtained by summing the genus counts for the 0-1 and 1-2 cm layers, taking into account total nematode abundances in each layer.

2.2.3 Nematode respiration rates

Total nematode biomass was calculated as the product of individual biomass (B, µg C ind^{-1}; calculated from the IRMS readings divided by the number of nematodes in the sample) and average density (determined from the field samples). Biomass-specific nematode respiration rates (R, µg C µg C^{-1} d^{-1}) were estimated using the formula by de Bovée and Labat (1993), divided by B:

$$R = \frac{0.0449 \times B^{0.8554} \times exp^{\ln Q10/10(T-20)}}{B}$$

with Q_{10} = 2, and T = temperature (°C). Total nematode respiration rates (µg C m^{-2} d^{-1}) were computed as the product of biomass-specific respiration rates (see equation above) with total nematode biomass (µg C m^{-2}). The mean nematode respiration rate in the 0-2 cm sediment layer for each station was obtained by averaging over experimental and control cores (GB1200: n = 4, GB1900: n = 7, WMed1900: n = 6).

2.3 Isotope enrichment experiments

2.3.1 ^{13}C-labeled substrates

The different methodologies employed in the experiments are summarised per station in Table 1. At seamount station GB1200, we used ^{13}C-glucose (99 % ^{13}C, $\delta^{13}C = 9 \times 10^6$ ‰) as a DOM substrate. At the slope stations, substrates were solutions of ^{13}C-glucose (15 % ^{13}C, $\delta^{13}C = 1.0 \times 10^4$ ‰) and ^{13}C-diatom DOM (11 % ^{13}C, $\delta^{13}C = 1.5 \times 10^4$ ‰). The glucose solution was prepared by dissolving a mixture of unlabeled and ^{13}C-labeled glucose (98-99 % ^{13}C) in 0.2 µm filtered seawater. The diatom DOM solution was extracted from ^{13}C-enriched axenic *Thalassiosira pseudonana*, which were cultured in the lab following Moodley et al. (2002). Diatom cells were thoroughly rinsed to remove residue ^{13}C-enriched bicarbonate, concentrated by centrifugation and then freeze-dried. Freeze-dried diatom cells were mixed with Milli-Q, vortexed and centrifuged (2000 G, 15 min), after which the supernatant was collected. Following three extractions, the collective supernatant was passed through a 0.2 µm polycarbonate filter (Millipore) to collect the passing diatom DOM in a glass tube. The carbon content of the diatom DOM (total and fraction of ^{13}C) was determined using an elemental analyzer coupled to a mass spectrometer. Thereafter, a fixed volume of diatom DOM was transferred to glass bottles, freeze-

dried and stored frozen until the initiation of the experiments. Prior to the experiment, 0.2 µm filtered seawater was added to re-dissolve the diatom DOM.

2.3.2 Experimental setup

2.3.2.1 Time-series experiment with ^{13}C-glucose at station GB1200

Upon retrieval, experimental cores from station GB1200 (i.d. 10 cm) were incubated in the dark at *in situ* bottom-water temperature (10 °C). The overlying bottom water in the cores was aerated with aquarium pumps. We injected three sediment cores with ^{13}C-glucose, equivalent to an addition of 60 mg C m^{-2} (resulting in a glucose carbon pore water concentration of 135.1 µM in the 0-5 cm sediment layer), at regularly spaced intervals covering the entire surface area using a 9 µl-syringe mounted on an extension rod. The syringe was inserted to 5 cm sediment depth and gradually emptied during retraction from the sediment to ensure a uniform depth distribution of the tracer. We sampled one core after 1, 7 and 14 days. The sediment of each core was sliced in 0-1, 1-2, 2-3, and 3-4 cm sections. From each 1-cm slice, sub-samples were taken for PLFA analysis (see 2.3.3), sedimentary TOC (i.e. POC + DOC), and nematode carbon isotope analysis, after which they were stored at -20 °C.

2.3.2.2 Experiments with ^{13}C-diatom-DOM and ^{13}C-glucose at stations GB1900 and WMed1900

At the 1900 m stations, experimental cores (i.d. 6 cm) were incubated for 7 days in the dark at *in situ* bottom-water temperature (GB1900: 4 °C, WMed1900: 13 °C). The overlying bottom water in the cores was aerated with aquarium pumps. We amended the cores with DOM carbon equivalent to an addition of ± 100 mg C m^{-2}, resulting in a pore water concentration of 568.5 µM in the 0-2 cm sediment layer. Injection of the ^{13}C-labeled substrates proceeded in the same manner as for the time-series experiment at GB1200, except that here, we injected the entire amount of DOM at 1 cm sediment depth. After 7 days, the top 2 cm of each core was removed and gently homogenized. From each 0-2 cm layer, 10 ml was analyzed for δ^{13}C of bacteria-specific PLFAs, while the remaining sediment was stored for nematode δ^{13}C analysis. Nematode δ^{13}C samples from GB1900 were stored at -20 °C, whereas those from WMed1900 were fixed in 4 % formalin.

2.3.3 Isotope and PLFA analysis

Sediment samples for carbon isotope analysis (only for station GB1200) were freeze-dried, ground with mortar and pestle, and acidified with dilute HCl to remove carbonates. Samples for nematode isotope composition were obtained by hand-picking 100-150 nematodes per sample (after elutriation with Ludox) and transferring them to a few drops of Milli-Q water in pre-combusted (450 °C for 3h) aluminium cups. Next, cups were dried overnight at 60 °C, closed and stored in a desiccator until analysis. Organic carbon content and isotope ratios of sediment and nematode samples were measured using a Thermo Flash EA 1112 elemental analyser coupled with a Thermo Delta V Advantage Isotope ratio mass spectrometer.

To determine bacterial biomass and ^{13}C uptake we extracted lipids from ca. 4 g of freeze-dried sediment using a modified Bligh and Dyer (1959) method (Boschker et al. 1999). The polar lipid fraction was obtained by fractionation on silicic acid, and derivatized using mild alkaline methanolysis to yield fatty acid methyl esters (FAMEs). The concentration and $\delta^{13}C$ values of these FAMEs were determined with a gas chromatograph - combustion interface - isotope-ratio mass spectrometer (GC-c-IRMS); a Hewlett Packard (HP) G1530 GC connected to a Thermo Delta+ IRMS via a Thermo III combustion interface. The $\delta^{13}C$ values of the bacteria-specific PLFAs i15:0 and ai15:0 were weighted with their respective concentrations to obtain a proxy for bacterial $\delta^{13}C$ (van Oevelen et al. 2006b). Since these two PLFAs make up roughly 11 % of all bacterial PLFAs (based on literature sources mentioned by Middelburg et al. 2000), and 5.6 % of the total carbon content in bacterial cells represents PLFA carbon (Brinch-Iversen and King 1990), the concentrations of i15:0 and ai15:0 allowed us to estimate bacterial biomass and DOM carbon incorporation into bacterial biomass (Middelburg et al. 2000).

Carbon isotope values were expressed in the δ notation relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C$ (‰) = $[(R_{sample}/R_{VPDB})-1] \times 10^3$, where R_{sample} is ^{13}C:^{12}C of the sample and R_{VPDB} is 0.0111802 (Post 2002). DOM carbon uptake was evaluated in terms of relative ^{13}C enrichment ($\Delta\delta^{13}C$ [‰] = $\delta^{13}C_{sample} - \delta^{13}C_{background}$), for the seamount station, and total DOM carbon uptake (µg C m^{-2}), for all stations. Total DOM carbon ($^{12}C + {}^{13}C$) uptake was calculated as ^{13}C uptake divided by the fractional abundance of ^{13}C ($^{13}F = {}^{13}C/ ({}^{13}C + {}^{12}C)$) in the labeled substrates. ^{13}C uptake was computed as the product of excess ^{13}C (E) and total carbon stock of the different compartments (bacteria, nematodes and sediments). E is the difference in the fractional abundance of ^{13}C between control ($^{13}F_{background}$) and enriched ($^{13}F_{sample}$) samples: $E = {}^{13}F_{sample} - {}^{13}F_{background}$. Total DOM carbon uptake by the nematodes was assumed to primarily reflect carbon assimilation, since the incubation periods (1, 7 or 14 days) largely exceeded previously reported defecation intervals of coastal nematodes (in the order of minutes, see also Moens et al. 1999c). To enable comparison with the slope stations, DOM carbon assimilation at the seamount station

(GB1200) was recalculated for the 0-2 cm layer sampled at day 7. This was done by taking the average $\delta^{13}C$ values of the bacterial PLFAs and nematodes over the 0-1 and 1-2 cm layer, weighted by their respective concentrations. For the slope stations, a difference in assimilation rates between glucose and diatom DOM of at least one order of magnitude was considered evidence for differential assimilation.

2.3.4 Evaluation of nematode feeding strategies

In the DOM amended cores, nematodes could acquire ^{13}C label by feeding on the DOM directly, or by feeding on other biota that had assimilated the added DOM, notably bacteria, or both. In this study, we considered two distinct, potential pathways of nematode label assimilation: either nematodes fed exclusively on DOM (referred to as the "DOM feeding strategy" in the remainder of the text), or they fed exclusively on bacteria ("bacterivorous strategy"). Nematode isotope dynamics in the time-series experiment at station GB1200 helped indicating which of these two hypothesized feeding strategies was most plausible, with direct DOM consumption evidenced by immediate, but transient ^{13}C-labeling and delayed, but longer-lasting ^{13}C enrichment indicative of bacterivory (van Oevelen et al. 2006b). In addition, we used two different approaches to quantify for all stations the contribution of bacterial carbon to the nematode diet, under a bacterivorous strategy, and that of DOM carbon to the nematode diet, under a DOM feeding strategy. If the dietary contribution was low or exceeded 100 %, the respective hypothetical feeding strategy was considered less likely.

Firstly, we compared the $\Delta\delta^{13}C$ values of nematodes ($\Delta\delta^{13}C_{nema}$) with the $\Delta\delta^{13}C$ of bacteria ($\Delta\delta^{13}C_{bact}$) and the $\delta^{13}C$ of DOM ($\delta^{13}C_{DOM}$) (Hall and Meyer 1998, van Oevelen et al. 2006b):

Contribution of bacterial carbon to the nematode diet, under a bacterivorous strategy (%):

$$\frac{\Delta\delta^{13}C_{nema}}{\Delta\delta^{13}C_{bact}} \times 100$$

Contribution of DOM carbon to the nematode diet, under a DOM feeding strategy (%):

$$\frac{\Delta\delta^{13}C_{nema}}{\delta^{13}C_{DOM}} \times 100$$

Given that the bulk of the DOM naturally present in sediment pore-waters is refractory (Burdige 2002), and our ^{13}C-labeled DOM substrates can be regarded as labile carbon sources, we assumed that the $\delta^{13}C$ value of the total labile DOM pool in the sediments equalled that of the ^{13}C labeled substrates.

In the second approach, we investigated if nematode assimilation of either food sources was sufficient to meet their carbon requirements. To this end, we compared the assimilation rates observed in the experiments with those required to maintain biomass-dependent respiration rates under a minimal nematode net growth efficiency of 0.6 (van Oevelen et al. 2006c). Total uptake of bacterial and DOM carbon by the nematodes was calculated as nematode ^{13}C uptake divided by the fractional abundance of ^{13}C in the bacteria and DOM, respectively.

2.3.5 Potential fate of DOM carbon

Our labelling approach allowed for the approximation of the DOM carbon flow through the bacteria and the potential flux through the nematodes. We considered the following pathways for DOM carbon (also see van der Meer et al. 2005, Soetaert and van Oevelen 2009):

Nematode uptake	=	Nematode assimilation + nematode respiration + nematode defecation
Bacterial uptake	=	Bacterial assimilation + bacterial respiration
Sediment uptake	=	adsorption to sediment particles + uptake by other benthic biota
Loss	=	upward and/or downward diffusion

We did not measure bacterial and nematode respiration, or nematode defecation, in the experiments. Therefore, we computed maximal values for these carbon flows on the basis of literature data on minimal efficiencies (and thus maximal loss through respiration and defecation) of bacterial growth (BGE = 0.05, van Oevelen et al. 2012) and on minimal growth (NGE = 0.6, van Oevelen et al. 2006c) and absorption efficiencies (AE = 0.3, van Oevelen et al. 2006c) of coastal nematodes. Using these DOM carbon flow estimates, we assessed the potential fate of DOM carbon within the sediments. Data on ^{13}C enrichment of sediment TOC (representing amongst other sediment adsorption, and label uptake by other benthic organisms) were only available for seamount station GB1200.

2.4 Data analysis

All statistical analyses were conducted in Primer v6 (Clarke and Gorley 2006) with the PERMANOVA+ add-on package (Anderson et al. 2008).

We checked for environmental differences between sediment depth layers at seamount station GB1200 (0-1, 1-2, 2-3, 3-4 cm) and between all stations for 0-2 cm sediment depth using Permutational Analysis of Variance (PERMANOVA). In addition, we performed a Principal Components Analysis (PCA) to visualize differences in environmental conditions between stations. Sedimentary total organic carbon content (TOC) and carbon: nitrogen ratios (C:N) were not included in the PCA because only one reliable replicate was available for these variables at station WMed1900. Sand and median grain size (MGS) were log-transformed to minimize right-skewness, whereas the sediment sorting coefficient (SC) was inverted to reduce left-skewness.

Benthic community structure was evaluated in terms of bacterial and nematode biomass, as well as nematode genus and feeding type relative biomass composition. For the time-series experiment at GB1200, nematode and bacterial biomass were compared between sediment layers and incubation time-steps with a two-way crossed Analysis of Similarities (ANOSIM) for unreplicated samples (assuming no or little change in vertical biomass profiles with time). Differences between stations in biomass within the surficial sediments (0-2 cm) were investigated using PERMANOVA. Nematode genus and trophic composition were compared between sediment depth layers (for the seamount station) and stations (0-2 cm layer) by means of PERMANOVA. For both generic and trophic composition, we used Bray-Curtis dissimilarities based on untransformed, relative biomass data. Where genus or trophic composition differed significantly between groups, a SIMPER analysis identified which genera or feeding types contributed most to the observed dissimilarity and which were typical of the different groups. A non-metric multidimensional scaling (nMDS) plot was constructed to visualize differences in genus composition between stations. The PERMDISP routine was employed to calculate the average dispersion in genus composition within stations. For some pairwise PERMANOVA tests, we interpreted the Monte Carlo P-value (P_{MC}) instead of the permutation P-value (P_{PERM}) because the number of possible permutations was < 100.

The results of the isotope enrichment experiments were not evaluated with statistical tests, because of the limited replication. Differences among stations in nematode biomass-specific respiration rates were tested for with PERMANOVA. When replicate samples were available, values were reported as mean ± standard error (SE).

3 Results

3.1 Environmental conditions

The environmental characteristics of all three stations are summarised per sediment layer (0-2 cm for all stations; 0-1, 1-2, 2-3, and 3-4 cm for station GB1200) in Table 2. At station GB1200, all environmental variables remained constant with sediment depth (PERMANOVA, $P_{PERM} > 0.05$, Table 3).

There was a clear distinction in environmental conditions within the 0-2 cm layer between seamount station GB1200 and the deeper slope stations (i.e. GB1900 and WMed1900; Fig. 2, Table 2 and 3). The eigenvector values indicated that all environmental variables contributed more or less equally to axis PC1 (explaining 88.1 % of the variation), which marked the separation between GB1200 and the other two stations. The most important contributors to the first PCA axis were MGS (-0.48) and SC (-0.47). The second PCA axis explained 9.0 % of the environmental variation and was mostly governed by TOM (-0.81).

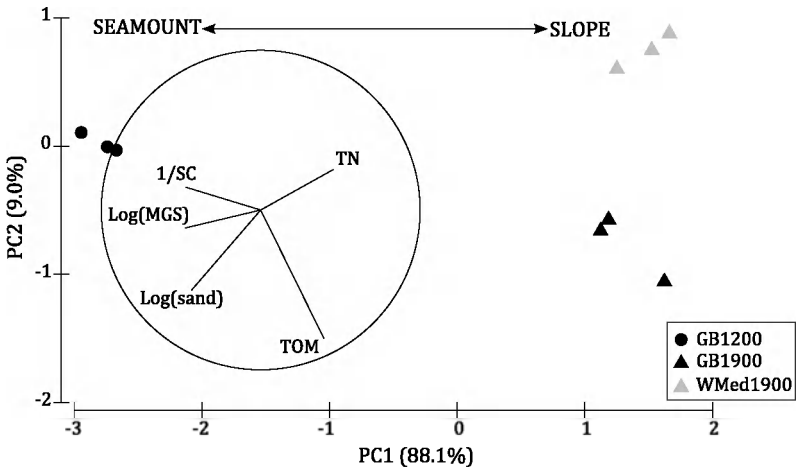

Fig. 2. *Principal components analysis (PCA) of normalized environmental variables. Median grain size (MGS) and sand were log-transformed to reduce right-skewness, whilst the inverse of the sediment sorting coefficient (SC) was taken to minimize left-skewness. TOM: total organic matter, TN: total nitrogen. Eigenvectors (lines) were superimposed.*

The univariate PERMANOVA analyses showed that GB1200 differed from stations GB1900 and WMed1900 in all measured environmental characteristics except for the C:N ratio, which was comparable between stations (Tables 2 and 3). GB1900 and WMed1900 displayed similar values for TN, TOC, C:N and sand content, but diverged with regard to MGS, TOM and SC (Tables 2 and 3).

3.2 Characterisation of the benthic community

3.2.1 Nematode and bacterial standing stock

At station GB1200, nematode and bacterial biomass did not change with sediment depth (Table 2; 2-way ANOSIM without replication, nematodes: P = 0.06, bacteria: P = 0.98) nor with incubation time (nematodes: P = 0.06, bacteria: P = 0.73). In all sediment layers, bacteria represented at least 92 % of total biomass (Table 2).

Bacterial standing stock in the top 2 cm of the sediment column differed significantly amongst stations (Table 2; PERMANOVA, Pseudo-F = 16.57, $P_{PERM} < 0.001$) with WMed1900 > GB1900 > GB1200 (pairwise PERMANOVA, GB1200-GB1900: $P_{PERM} < 0.05$, GB1200-WMed1900: $P_{PERM} < 0.01$, GB1900-WMed1900: $P_{PERM} < 0.01$). Nematode biomass also varied between stations (PERMANOVA, Pseudo-F = 5.43, $P_{PERM} < 0.05$), owing to the significantly higher standing stock at GB1900 compared to WMed1900 (Table 2; pairwise PERMANOVA, $P_{PERM} = 0.01$). Since bacteria dominated total standing stock of the small-sized biota investigated (Table 2), patterns in total biomass were driven by the bacteria. Consequently, total biomass peaked at WMed1900 (520.9 ± 73.0 mg C m^{-2}), declined at GB1900 (238.6 ± 27.1 mg C m^{-2}) and was minimal at GB1200 (108.0 ± 23.9 mg C m^{-2}) (PERMANOVA, Pseudo-F = 15.85, $P_{PERM} < 0.01$; pairwise PERMANOVA, GB1200-GB1900: $P_{PERM} < 0.05$, GB1200-WMed1900: $P_{PERM} < 0.01$, GB1900-WMed1900: $P_{PERM} < 0.01$). A PERMANOVA test (Pseudo-F = 20.03, $P_{PERM} < 0.001$) showed that the contribution of nematodes to the total standing stock of small-sized biota was significantly reduced at WMed1900 relative to the GB stations (Table 2; pairwise PERMANOVA: GB1200-GB1900: $P_{PERM} = 0.06$, GB1200-WMed1900: $P_{PERM} < 0.01$, GB1900-WMed1900: $P_{PERM} < 0.01$).

***Table 2**. Mean (SE) values of environmental variables and standing stock of sediment TOC (Sed), bacteria (Bact) and nematodes (Nema) per sediment layer at all stations. TN: total nitrogen, TOC: total organic carbon, C:N: molar carbon: nitrogen ratio, TOM: total organic matter, MGS: median grain size, sand: sand content, SC: sediment sorting coefficient. Environmental variables: n = 3, except for TOC and C:N at WMed1900, where n = 1. Stock concentrations were calculated from experimental cores, with n = 4 (GB1200), 7 (GB1900) or 6 (WMed1900). - : not determined.*

		TN	TOC	C:N	TOM	MGS	Sand	SC	Sed	Biomass (mg C m^{-2})		Biomass (%)	
		(%)	(%)	(-)	(%)	(µm)	(%)	(-)	(mg C m^{-2})	Bact	Nema	Bact	Nema
GB1200	0-1 cm	0.0186 (0.0020)	0.13 (0.02)	8.0 (0.3)	2.13 (0.14)	262.2 (15.1)	91.9 (1.6)	0.601 (0.045)	16397.8 (791.7)	52.4 (2.2)	4.4 (1.3)	92.4 (1.7)	7.6 (1.7)
	1-2 cm	0.0176 (0.0002)	0.11 (0.01)	7.6 (0.5)	2.15 (0.10)	246.1 (7.7)	91.9 (0.9)	0.586 (0.023)	15937.2 (827.9)	55.3 (20.9)	2.8 (0.2)	92.6 (1.6)	7.4 (1.6)
	2-3 cm	0.0202 (0.0015)	0.11 (0.01)	6.4 (0.5)	2.10 (0.05)	262.7 (8.7)	91.4 (0.4)	0.590 (0.005)	15192.9 (671.6)	32.9 (9.6)	1.7 (0.2)	93.8 (2.0)	6.2 (2.0)
	3-4 cm	0.0164 (0.0014)	0.09 (0.01)	6.5 (0.3)	1.96 (0.03)	231.9 (4.2)	91.3 (0.3)	0.616 (0.009)	14303.7 (527.8)	30.0(2.5)	0.8 (0.2)	97.4 (0.6)	2.6 (0.6)
	0-2 cm	0.0181(0.0011)	0.12 (0.02)	7.8 (0.4)	2.14 (0.06)	254.2 (10.0)	91.9 (1.1)	0.593 (0.031)	30914.3 (927.2)	100.7(22.6)	7.2 (1.4)	93.2 (0.4)	6.8 (0.4)
GB1900	0-2 cm	0.0649 (0.0058)	0.51 (0.04)	9.2 (1.1)	7.06 (1.07)	16.4 (1.0)	37.6 (7.6)	1.953 (0.097)	-	229.4 (27.2)	9.7 (1.2)	95.4 (1.0)	4.2 (0.9)
WMed1900	0-2 cm	0.0756 (0.0045)	0.80	11.2	4.59 (0.04)	11.5 (1.0)	19.1 (0.9)	1.651 (0.019)	-	516.8 (72.1)	4.1 (1.2)	99.2 (0.2)	0.8 (0.1)

Table 3. *Results of the one-way univariate PERMANOVAs testing for differences in environmental conditions at 0-2 cm sediment depth between stations. Significant differences (P < 0.05) are indicated in bold. When the main PERMANOVA test was not significant, we did not execute pairwise tests (-). TN: total nitrogen, TOC: total organic carbon, C:N: molar carbon: nitrogen ratio, TOM: total organic matter, MGS: median grain size, sand: sand content, SC: sediment sorting coefficient.*

	Main test			Pairwise tests		
				GB1200 vs. GB1900	GB1200 vs. WMed1900	GB1900 vs. WMed1900
	df	Pseudo-F	P_{PERM}	P	P	P
TN	2	50.90	**0.01**	**<0.01**	**<0.001**	0.22
TOC	2	92.32	**<0.001**	**<0.001**	**<0.001**	0.06
C:N	2	2.37	0.21	-	-	-
TOM	2	46.58	**<0.001**	**0.001**	**<0.001**	**<0.05**
MGS	2	562.62	**<0.01**	**<0.001**	**<0.001**	**<0.05**
Sand	2	72.73	**<0.01**	**<0.01**	**<0.001**	0.07
SC	2	142.43	**<0.01**	**<0.001**	**<0.001**	**<0.05**

3.2.2 Nematode community composition

The PERMANOVA analysis revealed no significant differences in nematode genus composition, based on relative biomass data, between sediment depth layers at station GB1200 (Pseudo-F = 1.32, P_{PERM} = 0.08) (Table 4). Potential bacterivorous nematodes (i.e. 1A + 1B + 2A) dominated total nematode biomass (99.4 ± 0.6, 96.1 ± 2.1, 66.3 ± 11.1 and 95.7 ± 2.8 % in the 0-1, 1-2, 2-3 and 3-4 cm layers) throughout the sediment column.

There was a clear distinction in genus composition within the 0-2 cm layer between the seamount station (GB1200) and the two slope stations (GB1900 and WMed1900) (main PERMANOVA, Pseudo-F = 4.53, P_{PERM} = 0.004; pairwise PERMANOVA, GB1200-GB1900: P_{MC} = 0.01, GB1200-WMed1900: P_{MC} = 0.04, GB1900-WMed1900: P_{MC} = 0.08). Fig. 3 indicates that the non-significant difference between the two 1900 m stations is caused by the high within-station variability in genus composition at WMed1900 (average dispersion is 38.6 and 21.8 for WMed1900 and GB1900, respectively). Given the relatively low P-value (< 0.10) and the low number of replicate samples (n = 3), which are quite variable, we find the evidence in support of the null hypothesis of no difference in composition not conclusive enough. Relative biomass and abundance data for the dominant genera are presented per station in Table 5, while the SIMPER results are shown in Table 6. Several genera typical of GB1200 (e.g. *Trefusia*, *Desmodora*, *Bolbolaimus*, *Microlaimus/Aponema*, *Leptolaimus*) contributed relatively little to nematode biomass at the other two stations (Table 6). In contrast, most genera typifying GB1900 and WMed1900 were also important at (one of) the other stations. Differences in community structure were most pronounced between GB1200 and WMed1900 (average dissimilarity: 76.0 %; Table 6). The genera mainly responsible for this disparity were *Sabatieria* (8.1 %) and

Bolbolaimus (7.8 %), which dominated nematode biomass at station WMed1900 and GB1200, respectively. GB1900 diverged from GB1200 (average dissimilarity: 71.4 %) because of the predominance of *Daptonema* and *Acantholaimus*, and the much lower biomass (and abundance) of *Desmodora* and *Bolbolaimus* at station GB1900.

The trophic composition of the nematode assemblages at each station is shown in Fig. 4. Even though the nematode assemblages at the two GB stations were generically distinct, they displayed a similar trophic structure (pairwise PERMANOVA, P_{MC} = 0.12). The feeding type composition at station WMed1900 was different from that at the GB stations (pairwise PERMANOVA, GB1200-WMed1900: P_{MC} < 0.01, GB1900-WMed1900: P_{MC} < 0.05). The SIMPER analysis indicated that the divergence between WMed1900 and the GB stations was primarily driven by the higher relative biomass of predators/scavengers (2B) at the former station (contribution to dissimilarity, WMed1900-GB1200: 2B: 44.1 %, WMed1900-GB1900: 48.8 %). Potential bacterivores prevailed at both GB stations (GB1200: 97.5 ± 12.5 %, GB1900: 91.9 ± 0.10 %). At station WMed1900, predatory/scavenging nematodes were dominant.

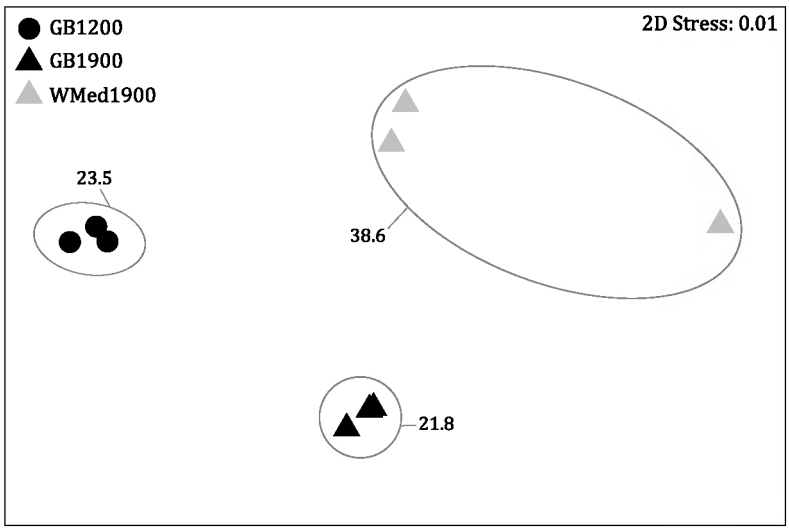

Fig. 3. *Non-metric multidimensional scaling (nMDS) plot of relative nematode genus biomass data for 0-2 cm sediment depth at all stations. The ellipses enclose samples from the same station. The numbers indicate the average within-station (or between-replicate) variability in genus composition, calculated via the PERMDISP routine in PRIMER.*

***Table 4**. Dominant genera (≥ 3 %) per sediment layer at station GB1200 in terms of average (SE) relative abundance and biomass, with an indication of feeding type (FT) based on buccal morphology according to the scheme by Wieser (1953).*

	0-1 cm			1-2 cm			2-3 cm			3-4 cm		
	Genus	FT	%	Genus	FT	%	Genus	FT	%	Genus	FT	%
Abundance	*Microlaimus/Aponema*	2A	12.4 (3.6)	*Richtersia*	1B	10.8 (4.2)	*Microlaimus/Aponema*	2A	9.2 (1.8)	*Desmodora*	2A	9.4 (5.3)
	Leptolaimus	1A	11.3 (1.4)	*Leptolaimus*	1A	10.4 (2.1)	*Daptonema*	1B	7.3 (2.9)	*Richtersia*	1B	9.0 (4.0)
	Desmodora	2A	10.3 (4.3)	*Desmodora*	2A	9.4 (2.0)	*Desmodora*	2A	6.8 (3.7)	*Daptonema*	1B	7.6 (1.7)
	Bolbolaimus	2A	8.1 (3.5)	*Bolbolaimus*	2A	8.0 (5.1)	*Richtersia*	1B	5.8 (3.1)	*Halalaimus*	1A	6.5 (1.1)
	Richtersia	1B	4.2 (2.1)	*Microlaimus/Aponema*	2A	6.6 (1.5)	*Metadesmolaimus*	1B	5.1 (0.8)	*Microlaimus/Aponema*	2A	6.5 (3.4)
	Metadesmolaimus	1B	4.0 (1.3)	*Metadesmolaimus*	1B	6.6 (0.1)	*Bolbolaimus*	2A	4.6 (2.3)	*Bolbolaimus*	2A	5.6 (4.7)
	Pselionema	1A	4.0 (1.3)	*Halalaimus*	1A	5.0 (2.1)	*Halalaimus*	1A	4.5 (0.2)	*Pselionema*	1A	5.1 (0.4)
	Acantholaimus	2A	3.8 (1.7)	*Trefusia*	1A	5.0 (0.8)	*Leptolaimus*	1A	4.4 (0.5)	*Prototricoma*	1A	5.1 (1.3)
	Halalaimus	1A	3.8 (0.8)	*Daptonema*	1B	4.7 (1.4)	*Syringolaimus*	2B	3.2 (0.8)	*Diplopeltula*	1A	3.8 (0.9)
	Daptonema	1B	3.7 (0.5)	*Acantholaimus*	2A	4.4 (0.8)						
Biomass	*Desmodora*	2A	13.4 (5.2)	*Trefusia*	1A	13.7 (3.2)	*Mesacanthion*	2B	9.5 (16.5)	*Metalinhomoeus*	1B	10.4 (9.0)
	Bolbolaimus	2A	11.6 (5.8)	*Bolbolaimus*	2A	11.1 (6.6)	*Bolbolaimus*	2A	8.3 (7.8)	*Desmodora*	2A	9.9 (6.4)
	Microlaimus/Aponema	2A	6.5 (2.4)	*Desmodora*	2A	10.2 (2.7)	*Oncholaimus*	2B	6.7 (11.6)	*Bolbolaimus*	2A	7.7 (9.7)
	Trefusia	1A	6.3 (3.3)	*Halalaimus*	1A	8.2 (3.2)	*Trefusia*	1A	6.1 (5.7)	*Richtersia*	1B	7.3 (5.5)
	Leptolaimus	1A	6.0 (0.8)	*Richtersia*	1B	7.4 (2.7)	*Desmodora*	2A	5.5 (6.3)	*Prototricoma*	1A	5.8 (2.7)
	Halalaimus	1A	4.7 (0.8)	*Leptolaimus*	1A	6.3 (1.3)	*Richtersia*	1B	5.3 (4.6)	*Trefusia*	1A	4.8 (4.5)
	Acantholaimus	2A	4.6 (1.9)	*Ammotheristus*	1B	5.8 (4.5)	*Camacolaimus*	2A	4.4 (5.4)	*Spirodesma*	2A	4.3 (7.5)
	Southerniella	1A	3.9 (1.8)	*Acantholaimus*	2A	5.6 (1.1)	*Viscosia*	2B	4.1 (4.7)	*Halalaimus*	1A	4.1 (2.4)
	Marylinnia	2A	3.8 (2.5)	*Metadesmolaimus*	1B	3.2 (0.2)	*Microlaimus/Aponema*	2A	3.3 (1.9)	*Sabatieria*	1B	3.7 (6.5)
	Richtersia	1B	3.5 (1.8)	*Microlaimus/Aponema*	2A	3.1 (0.8)	*Mesacanthoides*	2B	3.0 (5.2)	*Microlaimus/Aponema*	2A	3.7 (3.3)
	Pselionema	1A	3.1 (1.2)	*Syringolaimus*	2B	3.1 (1.5)	*Syringolaimus*	2B	2.9 (1.2)	*Linhomoeus*	2A	3.5 (6.1)
										Daptonema	1B	3.1 (2.0)

***Table 5**. Dominant genera (≥ 3 %) per station in terms of average (SE) relative abundance and biomass, with an indication of feeding type (FT according to the scheme of Wieser (1953).*

	GB1200			GB1900			WMed1900		
	Genus	FT	%	Genus	FT	%	Genus	FT	%
Abundance	*Leptolaimus*	1A	11.6 (0.7)	*Acantholaimus*	2A	13.4 (1.0)	*Metasphaerolaimus*	2B	12.5 (9.2)
	Microlaimus/Aponema	2A	10.3 (2.5)	*Daptonema*	1B	12.5 (2.1)	*Epsilonema*	1A	9.9 (9.9)
	Desmodora	2A	9.2 (3.1)	*Microlaimus/Aponema*	2A	7.5 (3.5)	*Acantholaimus*	2A	8.5 (1.2)
	Bolbolaimus	2A	8.3 (4.2)	*Diplopeltula*	1A	5.7 (1.8)	*Halalaimus*	1A	5.8 (0.6)
	Richtersia	1B	6.9 (2.8)	*Monhystrella*	1B	5.6 (0.8)	*Monhystrella*	1B	4.9 (1.6)
	Metadesmolaimus	1B	5.3 (0.6)	*Halalaimus*	1A	5.4 (0.9)	*Amphimonhystrella*	1B	3.8 (1.6)
	Daptonema	1B	4.2 (0.8)	*Leptolaimus*	1A	4.5 (2.2)	*Microlaimus/Aponema*	2A	3.1 (1.5)
	Acantholaimus	2A	4.0 (1.3)	*Desmoscolex*	1A	4.5 (0.5)	*Daptonema*	1B	3.0 (0.7)
	Halalaimus	1A	3.7 (0.4)	*Thalassomonhystera*	1B	3.6 (1.5)			
				Prototricoma	1A	3.2 (0.2)			
Biomass	*Bolbolaimus*	2A	11.8 (6.1)	*Acantholaimus*	2A	15.8 (1.8)	*Sabatieria*	1B	12.5 (7.0)
	Desmodora	2A	11.4 (3.7)	*Daptonema*	1B	14.2 (1.6)	*Epsilonema*	1A	11.1 (11.1)
	Trefusia	1A	9.6 (2.5)	*Halalaimus*	1A	8.9 (1.9)	*Metasphaerolaimus*	2B	9.1 (6.0)
	Leptolaimus	1A	6.8 (0.5)	*Diplopeltula*	1A	4.7 (1.8)	*Acantholaimus*	2A	6.5 (1.5)
	Halalaimus	1A	6.1 (0.7)	*Desmoscolex*	1A	4.3 (0.4)	*Halalaimus*	1A	4.1 (0.9)
	Richtersia	1B	5.5 (2.2)	*Sabatieria*	1B	4.1 (1.0)	*Mesacanthion*	2B	4.1 (4.1)
	Acantholaimus	2A	5.4 (1.8)	*Doliolaimus*	2B	3.5 (3.5)	*Richtersia*	1B	4.0 (2.9)
	Microlaimus/Aponema	2A	5.0 (1.2)	*Halichoanolaimus*	2B	3.4 (1.2)	*Cervonema*	1B	3.8 (0.5)
							Halichoanolaimus	2B	3.4 (1.7)
							Neochromadora	2A	3.0 (1.4)

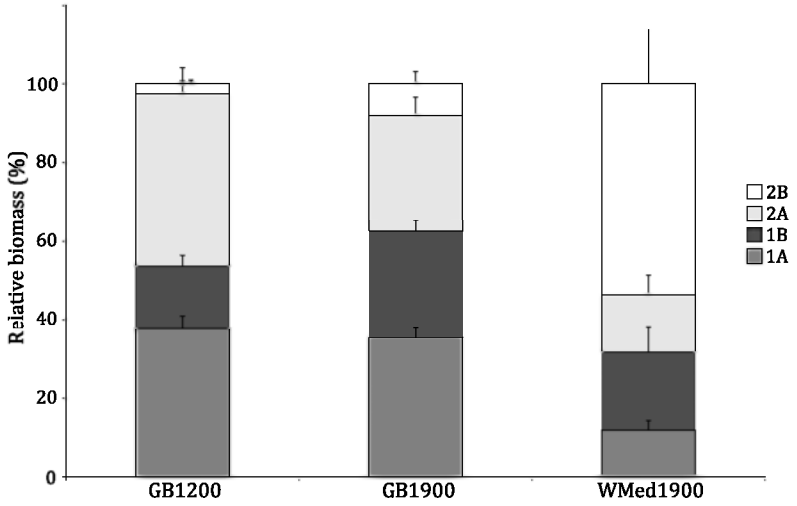

Fig. 4. *Nematode trophic composition (inferred from the relative biomass of feeding types) at 0-2 cm sediment depth per station. 1A: selective deposit feeders, 1B: non-selective deposit feeders, 2A: epistrate feeders, 2B: predators/scavengers. Error bars denote SE.*

Table 6*. List of the nematode genera that contributed most (≥ 3 %) to the similarity and dissimilarity within and between stations in terms of genus composition (based on relative biomass) at 0-2 cm sediment depth.*

	GB1200		GB1900		WMed1900	
GB1200	59.3 %					
	Desmodora	12.5 %				
	Trefusia	11.6 %				
	Leptolaimus	10.7 %				
	Halalaimus	9.0 %				
	Bolbolaimus	8.2 %				
	Microlaimus/Aponema	6.2 %				
	Acantholaimus	5.7 %				
	Richtersia	5.1 %				
	Metadesmolaimus	3.9 %				
GB1900	71.4 %		62.2 %			
	Daptonema	9.1 %	*Acantholaimus*	22.0 %		
	Bolbolaimus	8.3 %	*Daptonema*	20.3 %		
	Acantholaimus	7.2 %	*Halalaimus*	10.9 %		
	Trefusia	6.7 %	*Desmoscolex*	6.0 %		
	Desmodora	6.5 %	*Sabatieria*	4.8 %		
	Leptolaimus	4.0 %	*Diplopeltula*	4.5 %		
	Richtersia	3.9 %	*Halichoanolaimus*	3.2 %		
WMed1900	76.0 %		68.4 %		33.2 %	
	Sabatieria	8.1 %	*Trissonchulus*	11.3%	*Sabatieria*	14.9 %
	Bolbolaimus	7.8 %	*Daptonema*	9.3%	*Acantholaimus*	14.5 %
	Desmodora	7.5 %	*Acantholaimus*	6.9%	*Cervonema*	9.7 %
	Epsilonema	7.3 %	*Sabatieria*	6.9%	*Halalaimus*	9.1 %
	Metasphaerolaimus	6.0 %	*Richtersia*	4.5%	*Metasphaerolaimus*	7.2 %
	Trefusia	5.8 %	*Halalaimus*	4.2%	*Sphaerolaimus*	5.1 %
	Leptolaimus	4.2 %			*Halichoanolaimus*	4.8 %
	Richtersia	3.0 %			*Neochromadora*	4.6 %
					Spirobolbolaimus	3.0 %

3.3 Isotope enrichment experiments

3.3.1 Isotope dynamics at station GB1200

Carbon isotope signatures of nematodes in the control core of the time-series experiment (T0) were -21.5, -21.6, -21.7 and -23.1 ‰ in the 0-1, 1-2, 2-3 and 3-4 cm layers, respectively. Natural bacterial $\delta^{13}C$ values were -18.0 ‰ at 0-1, -21.1 ‰ at 1-2 and -17.5 ‰ at 2-3 and 3-4 cm sediment depth. The temporal dynamics in $\Delta\delta^{13}C$ of nematodes, bacteria and sediment TOC is illustrated in Fig. 5. Already one day after glucose injection (T1), both nematodes and bacteria had acquired some of the ^{13}C label; $\Delta\delta^{13}C$ values were 1.8, 17.8, 10.2 and 11.9 ‰ for bacteria and 0.8, 0.0, 4.6 and 19.9 ‰ for nematodes in the 0-1, 1-2, 2-3 and 3-4 cm layer. At T1, nematodes were depleted in ^{13}C relative to the sediment, but at T7 and T14, nematodes were more enriched than sediment TOC in all sediment depth layers ($\Delta\delta^{13}C_{nema} - \Delta\delta^{13}C_{sed}$ ranged between 1.5 and 256.0 ‰), indicative of selective feeding.

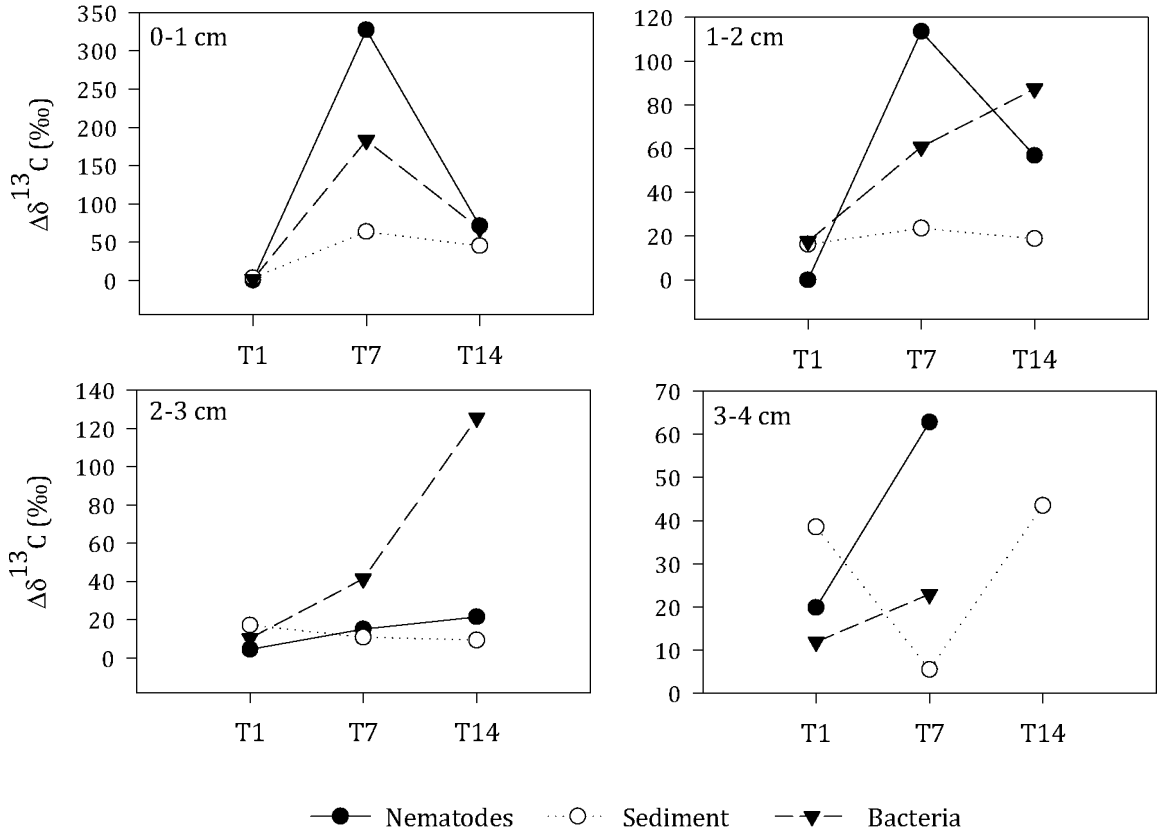

Fig. 5. *Temporal dynamics of the $\Delta\delta^{13}C$ values of nematodes, bacteria and sediment TOC per sediment depth layer at seamount station GB1200.*

At T7 (0-1, 1-2, 3-4 cm), in the 3-4 cm layer at T1, and in the 0-1 cm layer at T14, nematode $\Delta\delta^{13}C$ surpassed bacterial $\Delta\delta^{13}C$ values (Fig. 5). Consequently, the fraction of nematode carbon

derived from bacteria within these sediment horizons, as inferred from the $\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$ ratios and under the assumption that only bacteria consumed DOM directly, would exceed 100 %. This suggests nematodes were not feeding exlusively on bacterial carbon.

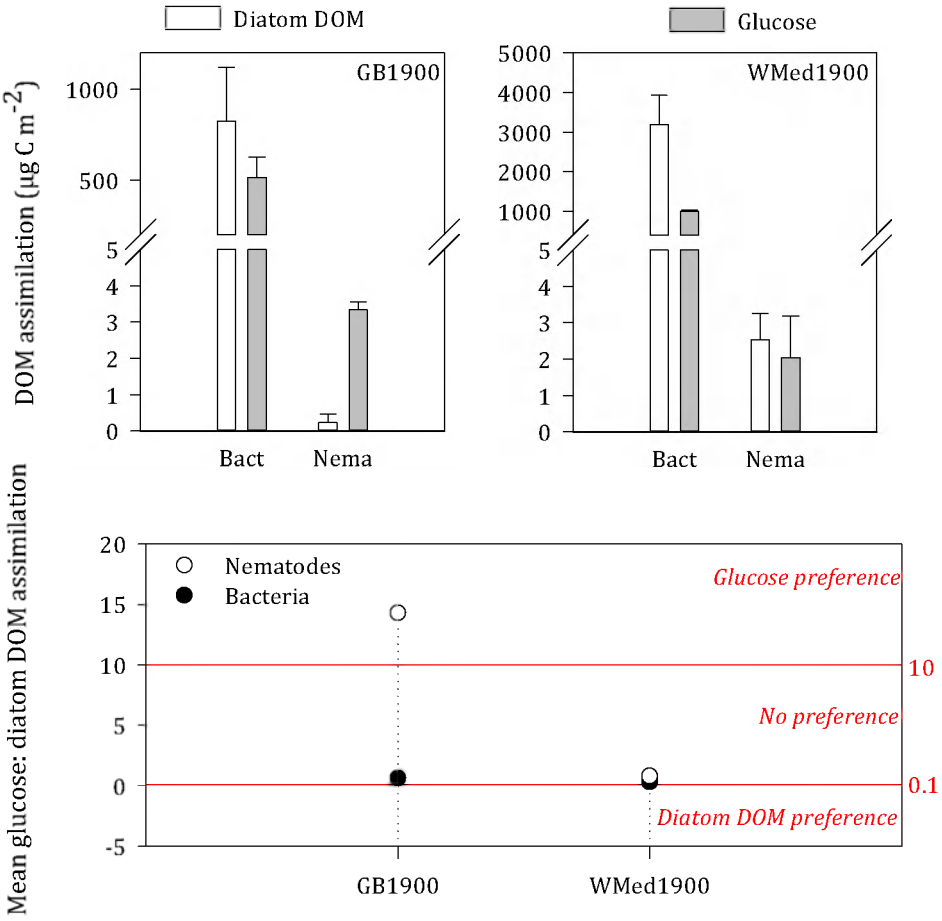

***Fig 6**. Diatom DOM vs. glucose assimilation by bacteria (bact) and nematodes (nema) at station GB1900 and Wmed1900 after 7 days in the 0-2 cm layer. Top: average assimilation of glucose and diatom DOM carbon. Error bars denote SE. Bottom: Dietary preference of glucose vs. diatom DOM. The lower red line denotes the situation where glucose assimilation is 10 times less than diatom DOM assimilation, while the upper red line indicates a 10-fold higher assimilation of glucose relative to diatom DOM. The zone between the red lines represents the case of no outspoken preferential assimilation.*

3.3.2 Glucose vs. diatom DOM assimilation

At both slope stations, bacteria showed no pronounced preference for diatom DOM or glucose, since the amount assimilated was of the same order of magnitude for both substrates (Fig. 6). At station WMed1900, nematodes took up comparable amounts of carbon in both treatments (Fig. 6). Thus, at WMed1900, nematodes did not discriminate between diatom DOM and glucose, assuming a DOM feeding scenario. Alternatively, assuming a bacterivorous strategy, nematodes

at WMed1900 consumed DOM- and glucose-feeding bacteria to the same extent. In contrast, at GB1900, nematode carbon uptake was almost 15 times higher in the glucose than in the diatom DOM cores (Fig. 6). Nematodes assimilated only 1.8 μg of diatom DOM carbon per m^2 in one diatom DOM core, while there was no uptake at all in the other core. These findings imply that nematodes at station GB1900 either preferred glucose over diatom DOM or preyed on bacteria and discriminated between bacteria feeding on diatom DOM and those feeding on glucose.

3.3.3 The importance of DOM and bacteria in the nematode diet

Table 7 contains data on the dietary significance of bacterial (under the bacterivorous strategy) and DOM carbon (under the DOM feeding strategy) for the different sediment layers at station GB1200 and at the different stations for the top 2 cm of the sediment column. Considering the isotope values for all incubation time-steps and sediment depth layers at seamount station GB1200, it was apparent that nematodes would have derived a very limited part of their carbon ($\Delta\delta^{13}C_{nema}/\delta^{13}C_{DOM}$ of max. 0.33 % at T7, 0-1 cm sediment depth) from glucose, feeding only on this food source. The share of bacterial carbon in the nematode diet ($\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$) surpassed that of glucose, and often exceeded 100 %. At T7 in the 3-4 cm layer, 273.3 % of nematode carbon would have originated from bacteria. The amount of glucose assimilated was never sufficient to balance respiration losses; it accounted for maximum 1.3 % (T1, 3-4 cm) of the necessary amount. Compared to direct DOM feeding, a bacterivorous strategy resulted in a greater fulfilment of carbon needs; at T1 in the 3-4 cm layer, nematodes had assimilated even more than what was required (119.7 %). The two methods we used to infer the share of DOM and bacteria in the nematode diet both showed highly variable values over the 0-4 cm sediment column at station GB1200, revealing no consistent trend with sediment depth.

***Table 7**. The contribution (%) of DOM (glucose or diatom DOM) and bacteria to the nematode diet at each station. For station GB1900, the results for the glucose and the diatom DOM cores are presented separately because of the pronounced difference in carbon assimilation between these two treatments. Values are averages (SE).*

			DOM		**Bacteria**	
			$\Delta\delta^{13}C_{nema}/\delta^{13}C_{DOM}$	assimilation/carbon demand	$\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$	assimilation/carbon demand
GB1200	T1	0-1 cm	0.0008	0.05	47.8	4.7
		1-2 cm	0.0000	0.00	0.0	0.0
		2-3 cm	0.0046	0.99	44.8	89.0
		3-4 cm	0.0199	1.34	166.9	119.7
	T7	0-1 cm	0.3276	0.57	178.8	43.7
		1-2 cm	0.1136	0.36	186.8	30.6
		2-3 cm	0.0152	0.04	36.6	3.5
		3-4 cm	0.0628	0.14	273.3	12.8
	T14	0-1 cm	0.0714	0.10	105.2	8.3
		1-2 cm	0.0569	0.07	65.1	6.0
		2-3 cm	0.0215	0.04	17.1	3.4
	T7	0-2 cm	0.2	0.5	141.2	36.9
GB1900	T7	0-2 cm	Glucose: 0.55 (0.02) Diatom DOM: 0.03 (0.03)	Glucose: 0.126 (0.008) Diatom DOM: 0.009 (0.009)	Glucose: 19.46 (0.06) Diatom DOM: 0.75 (0.75)	Glucose 1.691 (0.110) Diatom DOM 0.086 (0.086)
WMed1900	T7	0-2 cm	0.8 (0.3)	0.114 (0.029)	29.8 (16.5)	1.290 (0.349)

GB1200 was the only station where after 7 days nematodes ($\Delta\delta^{13}C_{nema}$ = 181.8 ‰) were more enriched in ^{13}C than bacteria ($\Delta\delta^{13}C_{bact}$ = 144.1 ‰) in the top 2 cm of the sediment (Fig. 5). At the two slope stations, nematodes ($\Delta\delta^{13}C_{nema}$ at GB1900: 0.3 ± 0.3 ‰ in the diatom DOM cores and 5.5 ± 0.2 ‰ in the glucose cores; $\Delta\delta^{13}C_{nema}$ at WMed1900: 8.4 ± 3.3 ‰) were labeled to a lesser degree than the bacteria ($\Delta\delta^{13}C_{bact}$ at GB1900: 34.0 ± 3.7 ‰, $\Delta\delta^{13}C_{bact}$ at WMed1900: 37.8 ± 7.7 ‰) after 7 days. For all three stations, the comparison of the relative enrichment of the nematodes and their potential food sources revealed that if nematodes would have fed on DOM directly, the fraction of carbon derived from this food source (always < 1 %) would have been much less than that from bacteria under a hypothetical bacterivorous strategy (ranging between 0.8 and 141.2 %; Table 7). In the 0-2 cm layer at station GB1200, the potential dietary contribution of bacteria was 700 times higher than that of glucose and amounted to 141.2 %. At the 1900 m stations, nematodes would have derived ~35 % more carbon from bacteria (GB1900: 0.8 ± 0.8 % in the diatom DOM cores and 19.5 ± 0.1 % in the glucose cores; WMed1900: 29.8 ± 16.5 %) than from DOM.

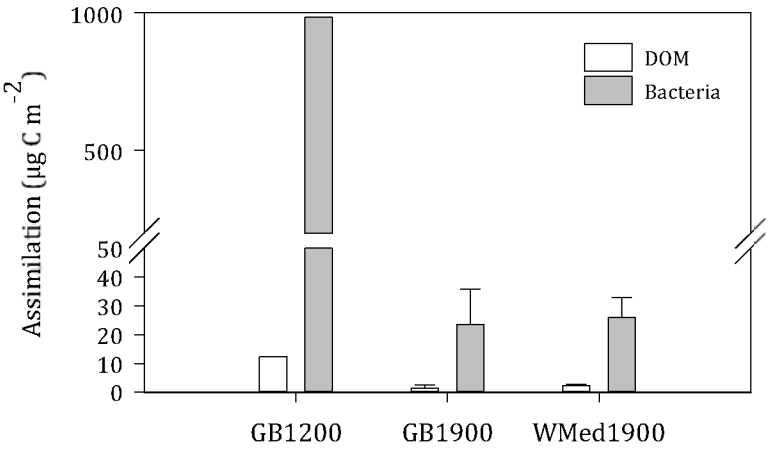

Fig. 7. *Nematode carbon assimilation under a DOM feeding (white bars) and a bacterivorous (grey bars) strategy at 0-2 cm sediment depth. DOM assimilation represents average assimilation of diatom DOM and glucose at stations GB1900 and WMed1900. Error bars denote SE.*

Nematode biomass-specific respiration rates in the 0-2 cm layer differed significantly among stations (PERMANOVA: P_{PERM} < 0.001, pairwise PERMANOVA tests: all P_{PERM} < 0.01) and were 0.0355 ± 0.0008 µg C µg C^{-1} d^{-1} at GB1200, 0.0244 ± 0.0003 µg C µg C^{-1} d^{-1} at GB1900, and 0.0491 ± 0.0018 µg C µg C^{-1} d^{-1} at WMed1900. The minimal amount of carbon that had to be assimilated by the nematodes to balance the amount respired over a period of 7 days at 0-2 cm sediment depth amounted to 2662.5 ± 444.6, 2469.3 ± 284.7 and 2000.2 ± 506.4 µg C m^{-2} for GB1200,

GB1900 and WMed1900, respectively. At all three stations, neither bacterial nor DOM assimilation fulfilled these carbon requirements (< 100 %; Table 7). The nematode assemblage at GB1200 (DOM: 12.36 µg C m^{-2}, bacteria: 983.9 µg C m^{-2}) incorporated more carbon than the nematodes at GB1900 (DOM: 1.51 ± 1.10 µg C m^{-2}, bacteria: 23.51 ± 12.36 µg C m^{-2}) and WMed1900 (DOM: 2.29 ± 0.58 µg C m^{-2}, bacteria: 25.89 ± 7.01 µg C m^{-2}), which displayed similar carbon assimilation values (Fig. 7). Hence, nematodes at the GB1200 station were most successful in meeting their carbon demands (feeding on DOM: 0.5 %, feeding on bacteria: 36.9 %; Table 7). In comparison, the DOM feeding and bacterivorous strategies at the two slope stations contributed at most 0.1 % and 0.1-1.7 %, respectively, to theoretical nematode carbon requirements. Since nematodes would have assimilated much more carbon when preying on bacteria (Fig. 7), bacterivory always resulted in a greater fulfilment of carbon demands than DOM feeding (Table 7).

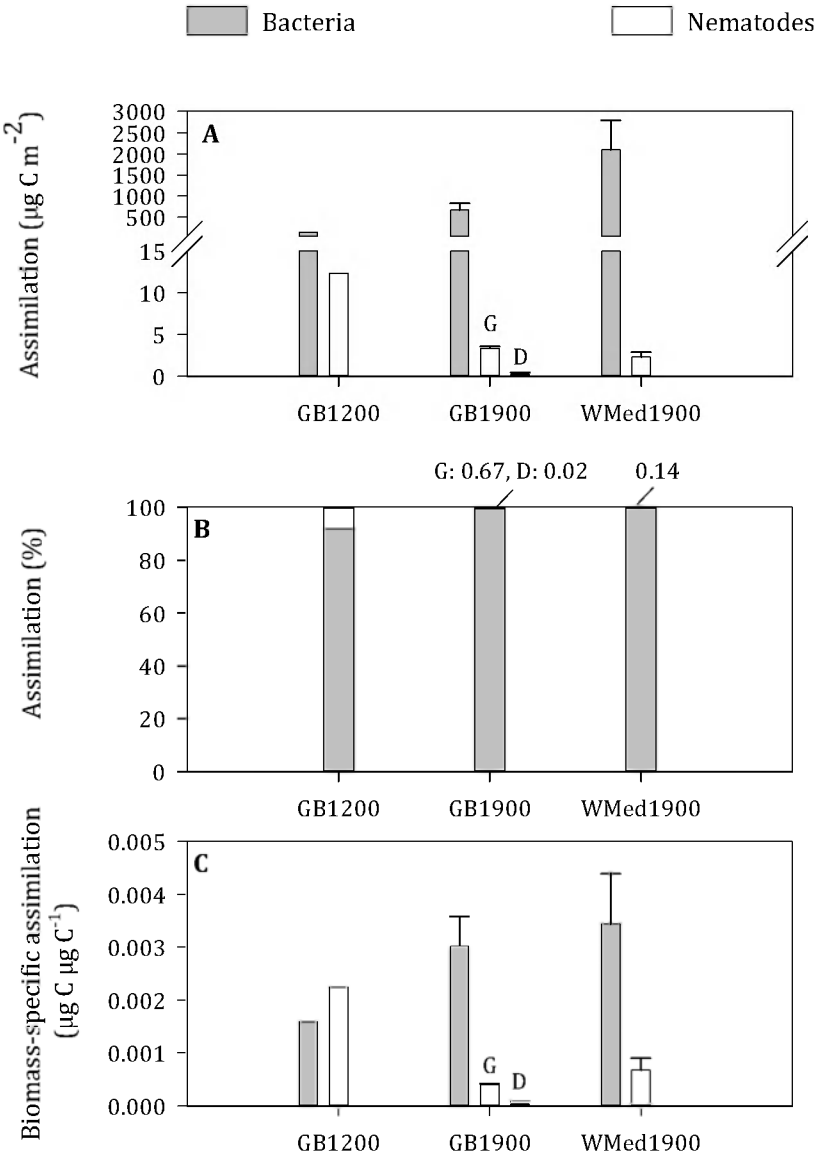

Fig. 8*. (A) Total assimilation, (B) assimilation partitioning and (C) biomass-specific assimilation by bacteria (grey bars) and nematodes (white bars) per station. For nematodes at station GB1900, carbon assimilation in the glucose (G) and the diatom DOM (D) cores are presented separately. At station WMed1900, the DOM assimilation averaged over both treatments is depicted. At GB1900 and WMed1900, the potential share of nematodes in total DOM carbon assimilation was negligible and therefore the actual values are given above the bars (B). Error bars denote SE.*

3.3.4 Potential fate of DOM carbon

To quantify maximal potential flows of added DOM carbon, we presumed nematodes fed directly and exclusively on DOM (i.e. the DOM feeding strategy), although the role of nematodes in the flux of DOM carbon was very limited. Since the nematode community at GB1900 assimilated

more carbon in glucose amended cores than in cores injected with diatom DOM, we plotted nematode carbon uptake at GB1900 for each treatment separately (Fig. 8). Bacteria assimilated more DOM-derived carbon compared to nematodes at all three stations (a factor of 10-1000), and were responsible for at least 92 % of total uptake (Fig. 8). However, per unit biomass, nematodes incorporated 1.5 times more glucose carbon relative to bacteria at station GB1200 (Fig. 8). At the two slope stations, biomass-specific assimilation was higher for bacteria than for nematodes. The contribution of nematodes to total DOM assimilation at station GB1900 was elevated in the glucose cores ($0.67 \pm 1.53 \times 10^{-5}$ %) compared to the diatom DOM cores ($0.02 \pm 3.26 \times 10^{-5}$ %). The share of nematodes in total DOM assimilation was ca. 10 and 50 times higher at seamount station GB1200 (8.06 %) relative to GB1900 (glucose: $0.67 \pm 1.53 \times 10^{-5}$ %, diatom DOM: $0.02 \pm 3.26 \times 10^{-5}$ %) and WMed1900 (0.14 ± 0.06 %), respectively.

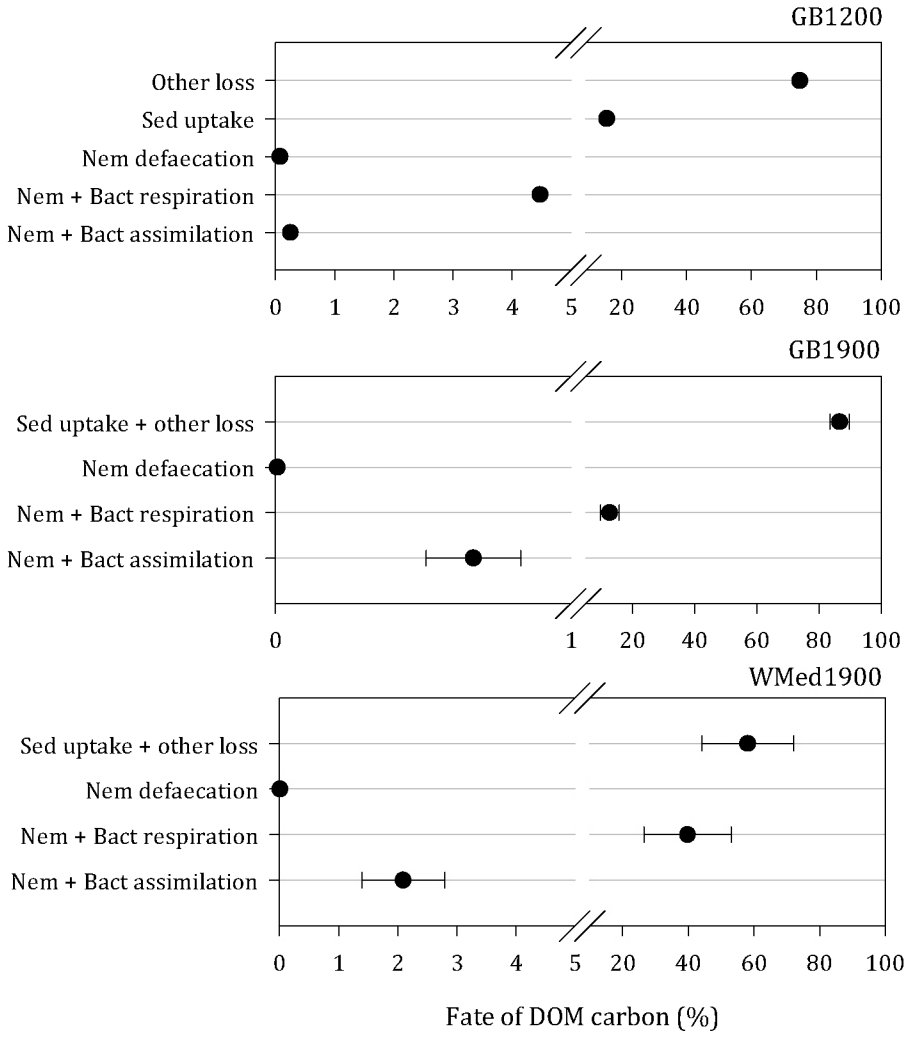

Fig. 9. *Fate of added DOM carbon at 0-2 cm sediment depth at the seamount (GB1200) and the slope stations (GB1900 and WMed1900). Nem: nematode, Bact: bacterial, Sed: sediment. Error bars denote SE. At station GB1200 there was only one replicate.*

Fig. 9 illustrates the fate of the added DOM carbon within the 0-2 cm sediment layer at all stations after 7 days. The majority of the DOM carbon added was not ingested by the bacteria and nematodes, and the fraction that was processed (i.e. assimilated, defecated or respired) by both biotic components declined according to WMed1900 (41.9 ± 14.0 %) > GB1900 (13.4 ± 3.1 %) > GB1200 (4.8 %). Assimilation was a minor pathway of DOM carbon processing and accounted at most for 0.26-2.10 % of the added DOM concentration. The fraction of added DOM carbon respired was 93.03, 94.92 ± 0.04 and 94.97 ± 0.01 % at GB1200, GB1900 and WMed1900, respectively. ^{13}C-enrichment of the sediment sampled at GB1200 showed that 20.67 % of this unprocessed carbon was present in the sediment pool. Respiration was the major fate of processed carbon at all stations (GB1200: 4.5 %, GB1900: 12.7 ± 3.0 %, WMed1900: 39.8 ± 13.3 %). The greatest fraction of DOM carbon was assimilated at station WMed1900 (2.1 ± 0.7 %), followed by GB1900 (0.7 ± 0.2 %), and then GB1200 (0.3 %).

4 Discussion

4.1 Benthic community composition in relation to environmental conditions

The environmental conditions at the seamount station (GB1200) in the Galicia Bank (GB) region contrasted strongly with those at the slope stations in the GB region (GB1900) and the western Mediterranean (GB1900). Compared to the slope, seamount sediments were coarser, sandier, and better sorted, indicating a high-energy environment (Van Weering et al. 2002, Duineveld et al. 2004).

As observed before (Moodley et al. 2005, Rex et al. 2006, Wei et al. 2010), bacteria dominated benthic biomass at all stations. Lowest bacterial biomass, however, was observed for the GB region, which does not fit with the proposed positive relationship between bacteria and the magnitude of seafloor POC flux (Deming and Yager 1992, Deming and Carpenter 2008). Primary productivity at the north-western Iberian margin (220 g C m^{-2} yr^{-1}; Joint et al. 2002), where the GB is located, exceeds that in the Algero-Provençal basin (153 g C m^{-2} yr^{-1}; Bosc et al. 2004). Bacterial standing stock was lowest at seamount station GB1200, indicating a negative impact of hydrodynamic activity as reported by Levin and Thomas (1989).

The hydrodynamic regime at the GB seamount affected nematode community composition, since seamount station GB1200 harboured a distinctive nematode genus composition, relative to GB1900 and WMed1900. In agreement, Vanreusel et al. (2010b) observed a clear difference in nematode community composition between multiple other slope and seamount sites, the latter

being dominated by genera like *Desmodora*, *Richtersia* and *Ceramonema*. These first two genera were also prominent at our seamount station. Vanreusel et al. (2010b) suggested that the dominance of these genera, which possess coarsely ornamented cuticles, may be related to the micro-structure of the sediments. The difference in geomorphology between seamount station GB1200 and the two slope stations (i.e. GB1900 and WMed1900) was not mirrored in a differential nematode trophic structure, since the communities at GB1200 and GB1900 had a similar feeding type composition. In contrast, seamount station GB1200 and slope station WMed1900 differed in trophic structure. Hence, the geomorphologic setting is merely one of several factors driving the trophic composition of nematode communities in deep-sea sediments.

4.2 Feeding strategies of deep-sea nematodes

Given the difference between stations in the amount of DOM injected and in injection mode (Table 1), the experimental results presented here should be interpreted with caution. Injecting the DOM substrate over 0-5 cm sediment depth (seamount station) should have resulted in higher label uptake in the deeper sediment layers, in comparison with the experiments where DOM was injected at 1 cm sediment depth. Our experimental set-up did not mimic the *in situ* hydrodynamic conditions of our stations. Under natural conditions, the relatively strong bottom-water currents at the seamount may induce pore-water advection, transporting DOM to greater sediment depths than in the diffusion-dominated sediments at the tranquil slope stations (Huettel and Gust 1992, Rusch et al. 2006). However, pore-water advection not only stimulates organic matter mineralisation through deeper penetration of labile organic matter, but also through the oscillation of redox conditions it generates (Franke et al. 2006), so *in situ* carbon uptake at the seamount may exceed that observed in the experiment. Furthermore, since the amount of natural labile DOM in the pore-waters is generally limited, observed assimilation rates represent potential uptake.

After 7 days, the seamount nematode assemblage was more enriched in ^{13}C than the sediments and the benthic bacteria. The higher isotopic enrichment of nematodes relative to the sediment suggests that nematodes feed selectively. This selectivity contrasts with the data from Guilini et al. (2010), who also injected ^{13}C-glucose in deep-sea sediments, but found that the $\delta^{13}C$ values of the nematodes were comparable to those of the sediment, and that both were lower than bacterial $\delta^{13}C$. Given the low specific enrichment and the absence of label accumulation by the nematodes, these authors surmised that nematodes assimilated the label associated with the added DOM from unselective feeding on sediment TOC. The higher nematode vs. bacterial

enrichment at the seamount points to the possibility of direct glucose ingestion by the nematodes. In support, glucose consumption has been demonstrated for shallow-water nematodes by Chia and Warwick (1969) and Montagna (1984). However, a bacterivorous strategy at seamount station GB1200 is also plausible if bacterial $\Delta\delta^{13}C$ peaked before day 1, or between day 1 and 7 after glucose injection. Guilini et al. (2010) observed maximal bacterial enrichment 2-4 days after glucose injection. Hence, considering a time lag between the consumption of labeled bacteria and the appearance of the ^{13}C label in nematode tissue, nematode feeding on these maximally enriched bacteria is plausible. Nonetheless, this is the first feeding study to show that direct DOM feeding in deep-sea nematodes is possible.

However, even though isotope dynamics indicated that direct DOM uptake is a plausible nematode feeding strategy at the seamount station, our data suggest that DOM is a relatively unimportant food source for the nematode community. The amount of nematode carbon derived from DOM, and the contribution of DOM assimilation to theoretical nematode carbon demands, was mostly less than 1 %. Bacteria constituted a potentially very important carbon source for the nematodes, since they were the source of a substantial part of the nematode carbon (which was occasionally well over 100 %) and bacterivory contributed much (but mostly < 100 %) to carbon requirements. However, the higher ^{13}C enrichment of nematodes vs. bacteria, and the fact that carbon requirements were not fulfilled, indicates that the diet of nematodes at GB1200 included, besides bacteria, other ^{13}C-labeled items like DOM. As at the seamount stations, the importance of DOM in the nematode diet at slope stations GB1900 and WMed1900 was negligible. Employing a bacterivorous strategy, nematodes derived a maximum of 20 % in the glucose amended cores at GB1900, and almost 30 % at WMed1900 of their carbon from bacteria. This together with the fact that the consumption of bacterial carbon contributed a maximum of 1.7 % to carbon requirements suggests that bacteria were not the main food source for nematodes at the slope stations.

The differences in assimilation rates between stations point to an influence of the abiotic environment and/or community composition on nematode feeding behaviour, though it seems not to be straightforward. The seamount station, where nematode carbon uptake was highest, diverged from the deeper slope stations (GB1900 and WMed1900) in terms of environmental characteristics and nematode genus composition. However, nematode trophic composition at both GB stations was similar. Compared to the slope sediments, the sandy seamount sediments had a low amount of organic matter and nitrogen, and low bacterial biomass (see Table 2). Thus, the rapid response of the seamount nematode assemblage may reflect an adaptation to the food-poor environment. In coastal sediments, nematodes also showed higher uptake of ^{13}C-enriched cyanobacteria (Urban-Malinga and Moens, 2006) and algae (Franco et al. 2008) in coarser than

in finer sediments. GB1900 and WMed1900, which were characterized by more similar environmental conditions, but had a distinct nematode genus and trophic composition, showed a comparable, low label uptake. It should be noted that the difference in timing, and thus in the amount of *in situ* organic matter deposition (see Figure 2B in Chapter 4), between the seamount (spring) and the slope experiments (autumn) may have contributed to the dissimilarity in assimilation rates. Pfannkuche et al. (1993) found enhanced benthic metabolic activity during sedimentation events in the deep sea, and thus the higher assimilation rates at the seamount may be related to an overall higher metabolic activity in spring.

Nematodes at station GB1900 showed higher assimilation rates in the glucose compared to the diatom DOM amended sediment cores, indicating a preference for either glucose (assuming a strict DOM feeding strategy) or bacteria that fed on glucose (bacterivorous strategy). Glucose is a simple sugar molecule (a pure carbon source), whereas diatom DOM consists of proteins, sugars and lipids (a source of carbon, nitrogen and phosphorous). Since nitrogen is a possibly limiting nutrient for deep-sea deposit feeders (such as the bulk of deep-sea nematodes) (Jumars et al. 1990), we expected the opposite, namely that nematodes would prefer diatom DOM over glucose. In coastal environments, glucose was shown to be a less attractive food source than fatty acids and diatom DOM (comprising amongst others fatty acids, lipids, and amino acids) for zebra mussels (Baines et al. 2005) and sponges (De Goeij et al. 2008), respectively. The alternative nematode ^{13}C enrichment pathway (i.e. bacterivory) implied that the composition of the bacterial assemblage feeding on diatom DOM and that feeding on glucose differed and nematodes favoured the latter. In support, shallow-water nematodes have been documented to prey selectively upon different bacterial strains (Moens et al. 1999a). Guilini et al. (2010) showed that the injection of different types of ^{13}C-enriched DOM targeted specific groups within the benthic bacteria. However, since bacteria and diatoms can have many PLFAs in common (Kelly and Scheibling 2012) and we did not possess data on the composition and the ^{13}C enrichment of the PLFAs in the added diatom DOM, we were unable to investigate whether the composition of the bacteria feeding on diatom DOM differed from those feeding on glucose. The contribution of DOM as a direct food source to deep-sea nematodes was deemed negligible and thus it seems more likely that nematodes fed selectively on the benthic bacterial community.

Guilini et al. (2010) and Gontikaki et al. (2011b) reported a limited contribution of bacterial feeding to nematode carbon requirements amounting to 0.1-5.1 % at the Arctic Hausgarten Site and 0.74 % in the sub-Arctic Faroe-Shetland channel, respectively. In comparison, bacterivory at the seamount station, where we used the same experimental methodology as Guilini et al. (2010), resulted in a greater fulfilment of carbon demands (ranging between 0 and 119.7 % over 0-4 cm sediment depth). In contrast, the contribution of bacterial assimilation to nematode

metabolic requirements at the slope stations (0.1-1.7 %) was as low as that found by Guilini et al. (2010) and Gontikaki (2011b). Because it is difficult to measure nematode respiration *in situ*, the present study and that of Guilini et al. (2010) and Gontikaki et al. (2011b), inferred nematode carbon demands from biomass-dependent respiration rates using the formula of de Bovée and Labat (1993). This equation was generated with data from several metabolic studies conducted in freshwater and coastal environments (see references in de Bovée and Labat 1993). Hence, the validity of the formula in the deep sea needs to be evaluated on the basis of *in situ* respiration measurements on deep-sea nematodes. Braeckman et al. (in press) showed that the respiration rate of an intertidal predatory nematode species decreased with lower oxygen availability. Assuming a similar oxygen-concentration dependence of respiration rates in the deep sea, these authors concluded that nematode respiration at the deep Arctic Hausgarten site, approximated following de Bovée and Labat (1993), was overestimated by a factor of two when the formula was not corrected for ambient, lower oxygen concentration. If nematode respiration is truly overestimated, nematode carbon assimilation observed in the experiments would have resulted in a greater fulfilment of carbon demands. Furthermore, we calculated minimal nematode carbon demands based on a minimal net growth efficiency (NGE) of 0.6, observed in coastal nematodes (see references in van Oevelen et al. 2006c). Bacteria in food-limited environments are characterized by low bacterial growth efficiency (BGE; Del Giorgio and Cole 1998), and it seems not unlikely for NGE to be related to food availability as well. Nevertheless, nematode carbon demands may be realistic and the potential food sources that were labeled in our experiments, i.e. benthic bacteria and labile DOM, may not be utilized to a large extent by nematodes. Modelling benthic carbon flows, Gontikaki et al. (2011b) and van Oevelen et al. (2012) concluded that semi-labile detritus was the main food source for deep-sea nematodes. To date, however, no experiments have been conducted to confirm this finding.

In environments with a considerable share of refractory organic matter, it was predicted by Jumars et al. (1990) for macro- and megafauna, and later demonstrated by Hall and Mayer (1998) for stream macro-invertebrates, that deposit feeders rely greatly on bacteria to meet their carbon and energy demands. Compared to macro- and megafauna, deposit-feeding nematodes possess (much) smaller buccal cavities, implying a more selective feeding behaviour. It follows that, in refractory settings, bacterivory might be more common in nematodes than in larger deposit feeders. The Algero-Provençal basin (WMed1900) experiences reduced primary productivity (153 g C m^{-2} yr^{-1}; Bosc et al. 2004) compared to the north-western Iberian margin (GB1900; 220 g C m^{-2} yr^{-1}; Joint et al. 2002). Thus, we expected a higher dependency of nematodes on bacterial carbon at WMed1900 relative to GB1900, but also at greater vs. shallower sediment depths at the seamount station. Our results indicated no difference in the trophic significance of bacteria to nematodes between the Mediterranean and northeast Atlantic

slope station, and between sediment depth layers at the seamount station. A similar lack of sediment depth-specific feeding behaviour was observed for coastal nematodes (van Oevelen et al. 2006b). The hypothesis of an elevated reliance on bacterial carbon in more refractory environments (Jumars et al. 1990, Hall and Mayer 1998) was thus not confirmed by our data.

The classification scheme of Wieser (1953) did not always support observed nematode carbon assimilation rates. Based on buccal morphology, potentially bacterivorous nematodes dominated both GB stations (> 90 % of total nematode biomass). However, the experimental results implied that bacteria were only potentially important for the seamount assemblage. Despite the divergent feeding type composition between GB1900 and WMed1900, carbon assimilation was comparable between these two stations. The former station had a higher proportion of non-selective deposit feeders (feeding type 1A), whereas the latter harboured more predators/scavengers (2B). Hence, assuming that predators/scavengers do not feed on bacteria and selective deposit feeders primarily do, buccal morphology actually implies a lower dependence on microbial food at the supposedly more refractory environment at the Mediterranean slope station. The lack of variation in carbon uptake with sediment depth at the seamount was in agreement with the feeding type classification, since genus (and hence feeding type) composition was comparable between sediment depth layers.

4.3 Fate of DOM carbon

In a feeding experiment with ^{13}C-enriched glucose at Sagami Bay (1453 m water depth), Nomaki et al. (2011) observed that at least half of the added glucose carbon was respired after 9 days. In the present study, we approximated respiration rates on the basis of biomass and growth efficiencies, and found a similar, dominant role for respiration in DOM carbon processing and only a little assimilation over a time span of 7 days at all three stations. The quantity assimilated by the bacteria and the nematodes (max. 2.1 % of the added DOM substrate) was in the same range as the values of Guilini et al. (2010) (1.6 - 5.1 %). Differences in carbon ingestion coincided with differences in total biomass between stations, and as such the amount of carbon processed by the benthic biota was determined by biomass. Presuming nematodes assimilated ^{13}C by feeding on DOM directly, bacteria had incorporated much more DOM carbon than nematodes after 1 week, as observed after 10 days for intertidal sediments by van Oevelen et al. (2006a). However, at the seamount station, nematodes assimilated more carbon per unit biomass than bacteria, in contrast with the slope stations and van Oevelen et al. (2006a). This result implies that the secondary production rate of nematodes over a period of 7 days exceeded that of bacteria, which is unprecedented.

Although Church (2008) stated that the bulk of bacterial production cannot be sustained by simple monomers (like glucose) only, bacteria did not assimilate much more diatom DOM than glucose in our experiments. In the present study, we used a fixed BGE (0.05) to approximate bacterial respiration rates. However, in reality, BGE varies with lability, nutrient and energy content of compounds, and between bacterial taxonomic groups (Del Giorgio and Cole 1998). Hence, even though the amount assimilated was comparable between the two substrates, the entire amount that was processed (i.e. respired + assimilated) by the bacteria may well not be. Owing to the variety of factors influencing BGE, it is difficult to predict the actual difference in BGE between the two types of DOM.

In experiments using labile POM as a tracer, a substantial part of the added carbon is often not processed by the benthic biota (Moodley et al. 2002, Woulds et al. 2007, 2009). In the present study, we provided labile DOM to the small-sized benthic biota, which we expected to be taken up more readily than POM. The main reasoning is that bacteria need to produce and excrete hydrolytic enzymes to break down POM before it can be transported through the cell wall. However, also in our experiments, the majority of the added substrates was left unprocessed by the bacteria and the nematodes (60-88 % on average). A similar observation was made by Nomaki et al. (2011). Sediment ^{13}C labelling at station GB1200 showed that about 20 % of this "lost" label was still present in the sediment, suggesting that a considerable part of the added glucose was adsorbed to the sediment particles (Henrichs and Sugai 1993, Keil et al. 1994, van Oevelen et al. 2006a). Given the coarse sediments at this station and the inverse relationship between adsorption and grain size (Mayer 1994), an even greater proportion of the added DOM would have been protected from mineralisation within the fine-grained sediments at WMed1900 and GB1900. However, sediment ^{13}C "uptake" also may have resulted from the DOM ingestion by other benthic biota like protozoans (including foraminiferans) and macrofauna. However, after 9 days, Nomaki et al. (2011) detected very little and no glucose assimilation by foraminiferans and copepods, and by meiofaunal-sized polychaetes and macrofaunal cumaceans, respectively. Notwithstanding the considerable ^{13}C enrichment of the sediment pool, most DOM carbon was lost from the sediment layer investigated at the seamount (0-4 cm) through diffusion to the upper water column or the deeper sediment layers.

4.4 Conclusions

This is the first nematode feeding study to demonstrate higher ^{13}C labelling of nematodes compared with bacteria and bulk sediment organic matter. Although the isotope dynamics in the time-series experiment showed that DOM and bacteria were both plausible nematode food

sources, the contribution to nematode secondary production and theoretical metabolic requirements revealed that bacteria represented a more important potential food source than DOM at all three stations. The higher carbon assimilation rates displayed by the seamount vs. the slope nematode assemblages may reflect an adaptation to the food-poor environment. Our data also showed that the trophic significance of bacteria to the nematode diet was related to the environment, though apparently not to the share of refractory organic matter, and the associated community composition. Furthermore, we noted that the carbon assimilation rates observed in the experiments were not always supported by the feeding type composition based on buccal morphology. Stations with a similar trophic structure showed large differences in nematode label assimilation, whilst stations with a dissimilar feeding type composition showed comparable uptake rates. The fraction of DOM carbon that was incorporated by the bacteria and nematodes depended on their total biomass, which was – as expected for deep-sea sediments – dominated by the bacteria. The majority of added DOM carbon was, however, not processed by the small-sized, benthic biota. The major fate of processed DOM carbon was respiration.

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Dave McCarthy

Chapter 4

Benthic-pelagic coupling: effects on nematode communities along southern European continental margins

In press, in Plos One:

Pape E, Jones DOB, Manini E, Bezerra TN, and Vanreusel A. Benthic-pelagic coupling: effects on nematode communities along southern European continental margins.

ABSTRACT - Along a west-to-east axis spanning the Galicia Bank region (northeast Atlantic) and the Mediterranean basin, a reduction in surface primary productivity and in seafloor flux of particulate organic carbon was mirrored in the *in situ* organic matter quantity and quality within the underlying deep-sea sediments at different water depths (1200, 1900 and 3000 m). Nematode standing stock (abundance and biomass) and genus and trophic composition were investigated to evaluate downward benthic-pelagic coupling. The longitudinal decline in seafloor particulate organic carbon flux was reflected by a reduction in benthic phytopigment concentrations and nematode standing stock. An exception was the station sampled at the Galicia Bank seamount, where despite the maximal particulate organic carbon flux estimate, we observed reduced pigment levels and nematode standing stock. The strong hydrodynamic forcing at this station was believed to be the main cause of the local decoupling between pelagic and benthic processes. Besides a longitudinal cline in nematode standing stock, we noticed a west-to-east gradient in nematode genus and feeding type composition (owing to an increasing importance of predatory/scavenging nematodes with longitude) governed by potential proxies of food availability (percentage of nitrogen, organic carbon, and total organic matter). Within-station variability in generic composition was elevated in sediments with lower phytopigment concentrations. Standing stock appeared to be regulated by sedimentation rates and benthic environmental variables, whereas genus composition covaried only with benthic environmental variables. The coupling between deep-sea nematode assemblages and surface water processes

evidenced in the present study suggests that it is likely that climate change will affect the composition and function of deep-sea nematodes.

1 Introduction

Apart from benthos dependent on chemosynthesis, deep-sea sediment communities ultimately depend for their dietary requirements on organic matter (OM) that is produced in the euphotic zone. The quantity that is produced varies among seasons and regions, and is determined by the physical properties and dynamics of the euphotic zone (Lutz et al. 2002). The particulate OM (POM) that is exported from the euphotic zone comprises phyto- and zoodetritus, in addition to bacteria, protozoans, faecal pellets (which mainly contain phytoplankton cells and gut bacteria) and inorganic compounds (Gage 2003). The processes through which POM is transferred to the deep-sea bottom are collectively termed "the biological pump". During its descent through the water column the POM particles are progressively broken down, and only a limited fraction (1 % on average) arrives at the deep-sea bed (Lutz et al. 2002). The fraction of exported POM that reaches the deep-sea sediments, or the efficiency of the biological pump, is determined by water depth, the sinking velocity (dependent on, amongst others, the degree of POM aggregation and the seawater mineral content) and the rate of decomposition of the POM (dependent on the pelagic food web structure and seawater temperature) (De La Rocha and Passow 2007). In addition, laterally advected water masses may transport sinking POM away from its point of origin (Gorsky et al. 2002, 2003, Zúñiga et al. 2007).

Various time-series studies documented an elevation in standing stock or metabolic activity, or both, of deep-sea benthic organisms in response to a phytodetritus pulse (Pfannkuche 1993, Pfannkuche et al. 1999, Duineveld et al. 2000, Galeron et al. 2001). Empirical evidence for the coupling between the pelagic and the deep-sea benthic realm comes from feeding experiments, showing rapid uptake of added phytodetrital matter by all benthic size-classes, from prokaryotes to megafauna (Witte et al. 2003a, Ingels et al. 2010, Jeffreys et al. 2011), including those living at abyssal depths (Aberle and Witte 2003, Witte et al. 2003b). Some authors, however, failed to detect a response of (some of) the deep-sea benthic biota under study (Gooday et al. 1996, Pfannkuche et al. 2000, Witbaard et al. 2000).

Meiofauna, a size-based invertebrate group dominated by nematodes, are a ubiquitous and dominant metazoan component of deep-sea sediments (Rex et al. 2006, Wei et al. 2010). Most proof for benthic-pelagic coupling stems from significant correlations between meiofaunal parameters and abiotic variables related to OM input. The magnitude of the flux of particulate

organic carbon (POC) to the seabed was documented to have a positive effect on meiobenthic abundance (Sommer and Pfannkuche 2000, Mokievskii et al. 2007). The concentration of chlorophyll-a (chl-a), and the sum of chl-a and its breakdown products (i.e. chloroplastic pigment equivalents or CPE) are commonly used to quantify the size of the fresh and total (fresh + degraded) phytodetrital pool, respectively, within the sediments (Boon and Duineveld 1996, Stephens et al. 1997). Sommer and Pfannkuche (2000), Soltwedel (2000), Neira et al. (2001), Tselepides et al. (2004), and Lampadariou et al. (2009) all described a positive association between meiobenthic or nematode standing stock and pigment concentrations. However, Danovaro et al. (1995) and Shimanaga et al. (2007) did not observe a relationship between meiofaunal abundance and pigment concentrations. As a consequence, it has been argued that not food quantity, but food quality structures deep-sea benthic assemblages (Danovaro et al. 1995). Other factors that were reported to correlate with deep-sea meiofaunal abundances or composition are granulometric characteristics (Tietjen 1984) and sedimentary organic matter content (Flach et al. 2002, Tselepides and Lampadariou 2004). However, the bulk of the OM within deep-sea sediments is refractory and thus organic matter content represents a poor measure of food availability (Soetaert et al. 1997).

We selected several study areas in southern Europe positioned along a west-east axis, which are characterized by differential trophic and oceanographic conditions, namely the Galicia Bank (GB) region, and several basins within the western and eastern Mediterranean. The GB is a seamount located on the north-western Iberian margin, which is marked by relatively high primary productivity (~220 g C m^{-2} yr^{-1}; Joint et al. 2002) owing to intense, wind-driven seasonal upwelling (McClain et al. 1986). However, in contrast to the non-seamount stations sampled in the GB region and the Mediterranean, the waters atop of the GB are hydrodynamically active (Duineveld et al. 2004), and as such bottom currents may interfere with OM deposition. Mediterranean deep-sea sediments represent an oligotrophic environment, because of the general nutrient depletion in surface waters combined with the high water temperature promoting the degradation of sinking OM (Tyler 2003). Within the Mediterranean, there is a well-established trophic divergence between the more productive western and the less productive eastern basin (Danovaro et al. 1999a, Turley et al. 2000, D'Ortenzio and Ribera d'Alcalà 2009). This gradient is generated by the higher nutrient input in the western Mediterranean owing to river runoff and the inflow of Atlantic surface water, and the outflow of relatively nutrient-rich Levantine Intermediate Water through the Strait of Gibraltar (also known as the inverse estuarine circulation) (Bergamasco and Malanotte-Rizzoli 2010). Nonetheless, there appears to be substantial regional heterogeneity in surface productivity within both the western and the eastern Mediterranean basin owing to hydrological features and river runoff (Estrada 1996, Bosc et al. 2004).

The aim of this study was to determine how differences in oceanographic and productivity regimes between our study areas are reflected in nematode community characteristics at different bathyal and abyssal water depths (1200, 1900 and 3000 m). Measures of surface productivity, seafloor POC flux and *in situ* OM quality and quantity were used to verify and describe the longitudinal trophic gradient. We hypothesized that the west-east decline in primary production and sedimentation results in lower standing stock and a gradient in the generic and trophic structure of the nematode assemblages towards the eastern Mediterranean. In addition, we assessed the importance of both POC flux and benthic environmental characteristics for the distribution and structure of nematode communities.

2 Materials and methods

2.1 Study area

The Galicia Bank (GB) is a seamount situated on the Iberian margin, about 200 km off the Galician coast. It is separated from the shallower parts of the continental margin by the Galicia Interior basin, which has an approximate depth of 3000 m (Fig. 1). The dome-shaped GB seamount has a relatively flat quasi-rectangular summit (between ca. 620 and 900 m water depth) which is covered by a thick layer of foraminiferal ooze and is bounded by steep scarps (Flach et al. 2002, Duineveld et al. 2004). Duineveld et al. (2004) measured high current velocities (5-30 cm s^{-1}) at 1 m above the GB summit. We collected samples at (1200 m; GB1200) and southeast of the GB seamount (1900 and 3000 m; GB1900 and GB3000, respectively) (Fig. 1). Hence, GB1200 is a seamount station, whilst the deeper stations were positioned on the slope. The oceanographic area in which these stations were located is termed the GB region throughout the manuscript.

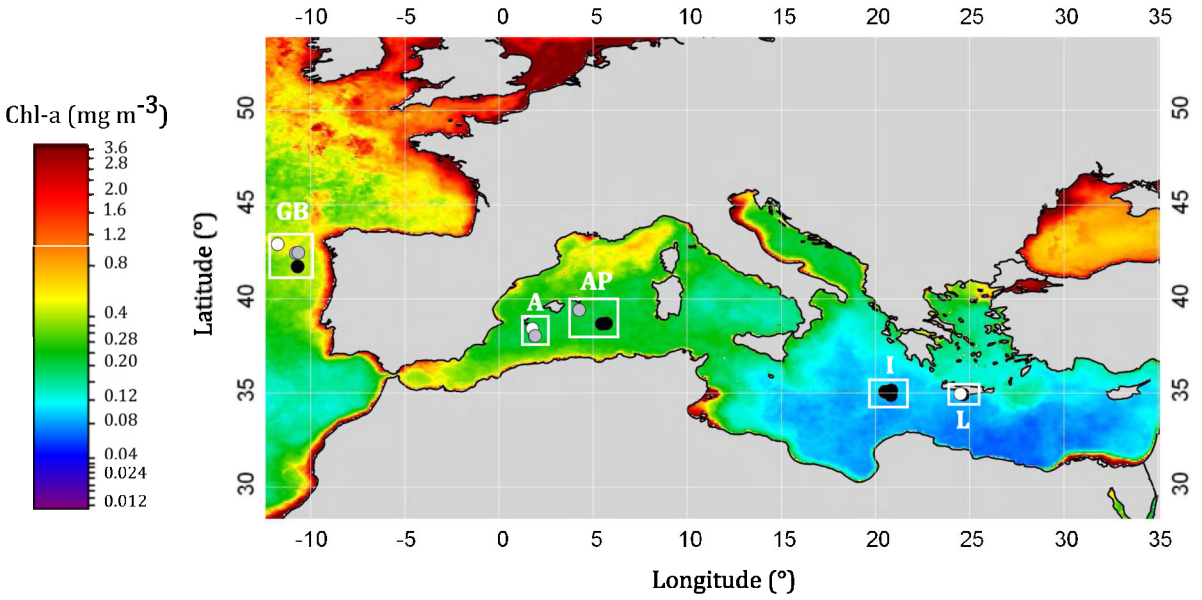

Fig.1. *Average 2008 chl-a levels of surface waters (Aqua Modis data, downloaded from Ocean Colour Web). Symbols indicate locations of sediment samples taken at 1200 (white), 1900 (grey) and 3000 m (black) water depth. The white rectangles enclose samples from the same region. GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin, L: Levantine basin.*

The Mediterranean Sea is split into the western and eastern Mediterranean basin by the Strait of Sicily. The western Mediterranean stations were situated in the Algerian ("A", A1200 and A1900) and in the Algero-Provençal ("AP", AP1900 and AP3000) Basin. The eastern Mediterranean samples were collected in the Ionian Sea ("I", I3000) and in the northern Levantine Basin ("L", L3000), offshore southern Crete. Sediment samples originating from each region were positioned on a west-to-east axis with an increase in longitude according to GB < A < AP < I < L (Fig. 1).

2.2 Sampling strategy

Sediment samples were gathered during various expeditions undertaken in the frame of the BIOFUN ("Biodiversity and ecosystem functioning in southern European deep-sea environments: from viruses to megafauna") project (Table 1). We initially planned to sample only with a multicorer, because this gear produces the least disturbed sediment samples (Bett et al. 1994). However, because of absence or mal-functioning of the multicorer, most of our samples comprised sub-samples from box cores taken with multicorer cores. Both Galeron et al. (2000) and Mokievskii et al. (2007) found no significant differences in meiobenthic abundances between multicorer and box corer samples, whereas Bett et al. (1994) observed twice as much

meiofauna in multicorer than in box corer samples. Samples taken in the same basin or region and at the same water depth during the same expedition were considered replicates of the same station. Note that the number of replicate deployments varied among stations (1-9; Table 1). The surface area of the sampling cores used was not constant, and measured 78.54, 10.18, 70.88, 56.45 and 69.4 cm^2 during the RV *Belgica*, *Urania*, *Pelagia* 2008, *Pelagia* 2009 and the *Sarmiento De Gamboa* expeditions, respectively. As a consequence, the total area sampled for nematode community analysis varied among stations (Table 1). All analyses were conducted on the top 0-1 cm of the sediment cores.

Table 1. *Sampling details with an indication of the region of origin (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin, L: Levantine basin), the period samples were collected in (month/year), station code (indicating basin and approximate water depth), the number of replicate samples for nematode community analysis (n; the total area of sediment sampled is shown in parentheses), granulometry and organics (OG) and pigment analysis (P) whereby samples taken from the same deployment (i.e. pseudo-replicates) are indicated in parentheses, water depth range, average geographical position (latitude and longitude expressed in decimal degrees; where the coordinates of replicates differed more than 1°, a range is given), sampling gear (BC: box corer; MC: multicorer), research vessel (RV; SDG: Sarmiento De Gamboa).*

			No of replicates							
Basin	**Station**	**Period**	**n (total area sampled, cm^2)**	**OG**	**P**	**Depth (m)**	**Lat**	**Long**	**Gear**	**RV**
GB	GB1200	Jun/08	3 (235.6)	3	0	1139 — 1141	42.9	-11.8	BC	*Belgica*
GB	GB1200	Oct/08	0	0	3	1155 — 1219	42.9	-11.8	BC	*Pelagia*
GB	GB1900	Oct/08	3 (212.64)	3	1 + (2)	1770 — 1896	42.4 — 42.5	-10.8 — -10.7	BC	*Pelagia*
GB	GB3000	Oct/08	3 (212.64)	3	3	3066 — 3072	41.7	-10.7	BC	*Pelagia*
A	A1200	Jun/09	3 (208.20)	3	3	1211 — 1214	38.4	1.8	MC	*SDG*
A	A1900	Jun/09	2 (138.80)	2	0	2004, 2016	38	1.9	MC	*SDG*
AP	AP1900	Nov/09	3 (169.35)	3	3	1582	39.4	4.3	MC	*Pelagia*
AP	AP3000	Jun/09	3 (208.20)	3	3	2841-2846	38.7	5.5 — 5.7	MC	*SDG*
I	I3000	Jun/08	7 (71.26)	7	9	2770 — 2808	34.9 — 35.1	20.5 — 20.8	BC	*Urania*
L	L1200	Jun/08	3 (30.54)	3	3	983 — 1143	35	24.6	BC	*Urania*
L	L3000	Jun/08	1 (10.18)	1	3	2458, 2647	34.9	24.5, 24.6	BC	*Urania*

2.3 Analysis of environmental variables

Granulometric analysis was conducted using a Malvern Mastersizer hydro 2000 G. Sediment fractions were classified according to the Wentworth scale (Wentworth 1922). Following freeze-drying and homogenization, samples were acidified with 1 % HCl. After acidification and drying, total organic carbon (TOC) and nitrogen (TN) content were measured using a Flash EA 1112+ MAs 200 elemental analyser (Thermo Interscience). Total organic matter (TOM) content was determined after combustion of the sediment samples at 550 °C.

Chlorophyll-a (chl-a) and phaeopigment analyses were carried out according to Lorenzen and Jeffrey (1980). Pigments were extracted (12h at 4 °C in the dark) from triplicate superficial (0-1cm) sediment samples (± 1 g), using 5 ml of 90 % acetone. Extracts were analysed fluorometrically to estimate chl-a, and after acidification with 200 ml 0.1 N HCl, to estimate phaeopigments. Chloroplastic pigment equivalents (CPE) constitute the sum of chl-a and phaeopigments. The ratio of chl-a and phaeopigments (chl-a:phaeo) was considered as a proxy for the "freshness" of the phytodetrital input.

Besides benthic environmental variables, we analyzed environmental data related to the pelagic realm. Net primary production (NPP) values were extracted from the Vertically Generalised Production Model (VGPM; resolution: 1°) described by Behrenfeld and Falkowski (1997) and downloaded from http://www.science.oregonstate.edu/ocean.productivity/. The VGPM estimate of NPP values was based on satellite measurements of sea surface temperature (SST), surface water chl-a concentrations, and photosynthetically active radiation (PAR). Because only monthly data are available on NPP and in our study area variation in PAR and SST is negligible, we superimposed annual composite chl-a concentrations (Level-3 Aqua Modis data from 2008 with a resolution of 9 km; http://oceancolor.gsfc.nasa.gov) on a map displaying the locations of our samples to illustrate the heterogeneity in NPP. To this end we used the freely available HDF view, SAGA (System for Automated Geoscientific Analyses) and Quantum GIS (QGIS, v 1.7.4.) applications. Data on the particulate organic carbon (POC) flux to the seafloor were approximated on the basis of water depth and seasonal variation in NPP, calculated as the standard deviation divided by the mean of monthly NPP values, according to Lutz et al. (2007).

NPP and POC flux to the seafloor were calculated for each sample location listed in Table 1. Owing to the 1° resolution of the input data for the VGPM model, replicate stations were often assigned equal NPP values and differences in seafloor POC flux were simply as a result of the variability in measured water depth.

2.4 Nematode community analysis

The sediment samples (0-1 cm sediment depth) fixed in seawater-buffered 4 % formalin were washed over a 32 µm mesh sieve and the meiofauna extracted from the sediment by Ludox centrifugation (Heip et al. 1985). Meiofauna was then sorted, enumerated and identified at higher taxonomic level. Where possible, ± 100 nematodes were hand-picked from each sample and identified to genus level. Since it was difficult to distinguish between *Microlaimus* and *Aponema*, specimens belonging to one of these genera were allocated to a *Microlaimus/Aponema* complex. Nematodes were grouped into four feeding types on the basis of the morphology of their buccal cavity *sensu* Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate feeders and scavengers/predators (2B). Additionally, we measured length (L; µm) and maximal width (W; µm) for each nematode to estimate individual wet weight (WW) using Andrassy's formula (1956), adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm^{-3}; µg WW ind^{-1} = L × W^2/1500000). Individual biomass (µg C ind^{-1}) was then estimated as 12.4 % of WW (Jensen 1984). Total nematode biomass (µg C 10 cm^{-2}) in each sample was calculated as the product of nematode density (ind. 10 cm^{-2}) and the arithmetic mean of individual biomass values.

2.5 Data analysis

Longitudinal and bathymetric monotonic (linear + non-linear) trends in environmental (seafloor POC flux, phytopigments, MGS, mud, TOC, TOM, TN and C:N) and univariate nematode variables (density, individual and total biomass and relative abundance of feeding types) for a given depth or longitude were investigated by means of partial Spearman rank correlations. The strength and direction of longitudinal and bathymetrical gradients were indicated by $r_{long|depth}$ (correlation with longitude, given depth) and $r_{depth|long}$ (correlation with depth, given longitude), respectively. Fourth-root transformed relative nematode genus abundances were subjected to distance-based linear modelling (DISTLM) to determine (1) whether spatial variation in genus composition was mostly owing to longitude or to water depth (shown by the marginal tests) and if (2) depth/longitude contributed to the explained variation, given longitude/depth (checked by the conditional tests). Genus composition data were visualized using non-metric multi-dimensional scaling (nMDS). To assess which genera described most of the longitudinal variation in community structure, we employed a BEST analysis using the fourth-root transformed genus abundances and the Bray-Curtis resemblance matrix based thereon, at each approximate depth (1200, 1900 or 3000 m). This type of analysis can be seen as a generalization of the SIMPER routine as it searches for a subset of genera that can account for the whole continuous pattern

(Clarke and Warwick 2001a). A SIMPER analysis was conducted to identify the nematode genera that discriminated most between the seamount and the slope stations.

To determine the importance of the abiotic environment to nematode standing stock and composition, we conducted Spearman rank correlation and RELATE analysis (by means of Spearman rank correlations), respectively. Because pigment data were mostly obtained from different deployments than nematode and other environmental variables (Table 1), replicate environmental and nematode values were averaged per station. As a measure of within-station variability in nematode genus composition we used relative dispersion obtained through the MVDISP routine (Clarke and Warwick 2001a), which we also subjected to correlation tests with all environmental variables.

Univariate correlation tests were executed in R v 2.15.0 (R Core Team 2012). Partial correlations were obtained with the R package ppcor (Kim 2011). All other analyses were done in PRIMER v6 with the PERMANOVA+ add-on (Clarke and Gorley 2006, Anderson et al. 2008). Because GB1200 was the only seamount station amongst all slope stations, it was omitted from the analysis of bathymetric and longitudinal trends in benthic (environmental and nematode) parameters and the correlation test between environmental and nematode community descriptors. The data on nematode community structure and benthic environmental variables at the seamount station were presented separately from those at the non-seamount stations (3.2). Data were expressed as means ± standard error (SE).

3 Results

3.1 Longitudinal and bathymetrical trends in NPP and seafloor POC flux

There was a significant reduction in NPP along the west-to-east axis from the GB region to the eastern Mediterranean basin (Fig. 2A; Spearman rank: $r = -0.89$, $P < 0.001$). Because they were distanced by 1° longitude or more, some samples from the same basin, either from equal (i.e. the 3000 m samples from the Algero-Provençal and Ionian basin) or different water depths (1200 and 1900 + 3000 m samples from the GB region and 1900 and 3000 m samples in the Algero-Provençal basin), displayed differential NPP (Fig. 2). Nevertheless, on average, NPP ranged between 716.2 g C m^{-2} yr^{-1} at the GB region and 384.2 g C m^{-2} yr^{-1} in the Levantine Basin. Within the western Mediterranean, the Algerian Basin (688 g C m^{-2} yr^{-1}) exhibited elevated NPP relative to the Algero-Provençal Basin (540.3 - 572.5 g C m^{-2} yr^{-1}). In the eastern Mediterranean basin,

there was a small drop in NPP between the Ionian (400.0 g C m^{-2} yr^{-1}) and the Levantine Sea (384.2 g C m^{-2} yr^{-1}).

Seasonal variability in NPP at the GB region (0.43) was more than twice that in the Mediterranean, where values slightly increased from west to east with 0.16, 0.18, 0.20 and 0.19 in the Algerian, Algero-Provençal, Ionian and Levantine basin, respectively (see also Fig 2B). The GB1200 station experienced maximal NPP in April, while the deeper stations in the GB region showed an additional, but less pronounced NPP peak in August (Fig. 2B). The Mediterranean stations experienced two NPP maxima per year; one in March and another one in July.

For a given water depth, seafloor POC flux related negatively with longitude (Fig. 3A; Table 2). When longitude was fixed, POC flux declined along the bathymetrical axis.

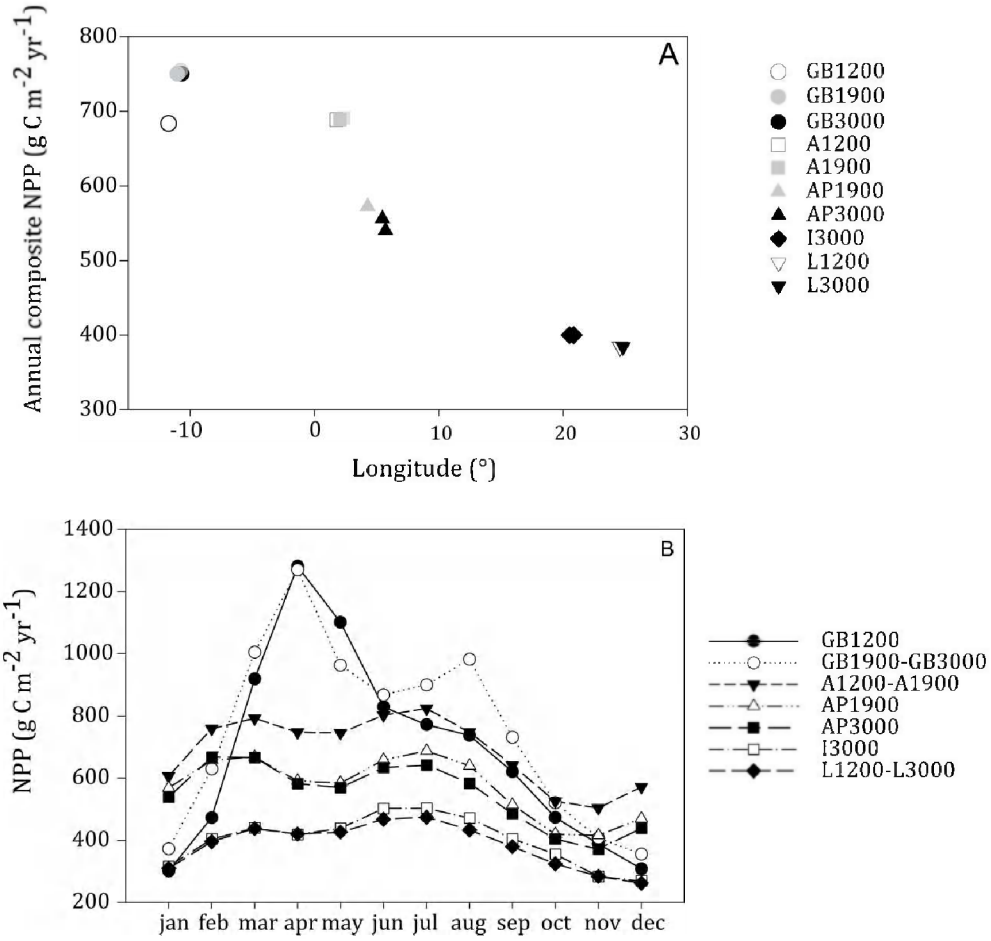

Fig. 2. *(A) Annual composite NPP in function of longitude. Symbols represent stations, indicating basin (shape) and water depth (colour). GB: Galicia Bank region, A: Algerian Basin, AP: Algero-Provençal Basin, I: Ionian Basin, L: Levantine Basin. (B) Monthly variation in NPP. Each line represents NPP for stations located less than 1° longitude apart.*

3.2 Benthic environmental and nematode community characteristics of the seamount station

Even though we obtained the highest seafloor POC flux values for GB1200 (Fig. 3A), this station was characterized by the lowest phytopigment concentrations of all (chl-a: 0.0070 ± 0.0006 μg g^{-1}, CPE: 0.441 ± 0.035 μg g^{-1}) (Fig. 3B-C). In addition, we observed divergently low values for TN (0.045 ± 0.002 %; Fig. 3G), TOC (0.13 ± 0.02 %; Fig. 3H) and TOM (2.13 ± 0.14 %; Fig. 3J). The samples collected at GB1200 contained low numbers of nematodes (96.9 ± 27.7 ind. 10 cm^{-2}) relative to the 1200 m station in the western Mediterranean (203.1 ± 5.0 ind. 10 cm^{-2}) and the deeper stations in the GB region (GB1900: 213.5 ± 13.7 ind. 10 cm^{-2}; GB3000: 180.0 ± 54.7 ind. 10 cm^{-2}) (Fig. 4). Fig. 5 shows that the nematode generic composition at the seamount station diverged from that at the non-seamount stations. The SIMPER analysis indicated that this divergence (average dissimilarity: 60.4 %) was partly driven by the higher relative abundance of *Bolbolaimus*, *Desmodora*, *Metadesmolaimus*, and *Microlaimus/Aponema* and the absence of *Sphaerolaimus* at GB1200 (Table 3). *Bolbolaimus* was one of the 13 genera that were restricted to station GB1200. Similar to the non-seamount stations, the seamount station was dominated by deposit-feeding nematodes (feeding types 1A + 1B; 54.9 ± 5.3 %). Of all stations, GB1200 had the highest fraction of epistrate feeders (2A; 44.2 ± 5.5 %) and the lowest fraction of predatory/scavenging species (2B; 0.88 ± 0.45 %; Fig. 6).

3.3 Benthic environmental and nematode community characteristics of the non-seamount stations

3.3.1 Longitudinal and bathymetric trends in benthic environmental variables

The partial Spearman rank correlation coefficients describing longitudinal and bathymetrical trends at a fixed depth and longitude, respectively, are presented in Table 2. Station AP3000 displayed relatively low POC deposition, albeit elevated sedimentary phytopigment levels (chl-a: 0.3205 ± 0.0108 μg g^{-1}, CPE: 6.005 ± 0.324 μg g^{-1}) in comparison with the stations in the GB region and the Algerian basin, and with AP1900. We found a significant longitudinal decline in chl-a and CPE levels (Fig. 3B-C), which was more pronounced (i.e. more negative value of rlong|depth) when station AP3000 was excluded from the analysis. Mud content (Fig. 3F) and MGS (Fig. 3E) increased and decreased, respectively, from west to east. The percentage of TOM showed a positive relation with longitude and with water depth (Fig. 3J). Sedimentary TOC (Fig. 3H), TN (Fig. 3G) and consequently C:N (Fig. 3I) values did not change with depth or longitude.

Table 2. *Partial Spearman rank correlations with longitude, given depth ($r_{long|depth}$), and depth, given longitude ($r_{depth|long}$) for environmental and nematode community variables. Environmental variables: POC flux (annual particulate organic carbon flux to the seafloor), chl-a (chlorophyll a), CPE (chloroplastic pigment equivalents), chl-a:phaeo (chlorophyll a: phaeopigments ratio), MGS (median grain size), mud, TOM (% of total organic matter), TOC (% of total organic carbon), TN (% of total nitrogen), C:N (molar carbon: nitrogen ratio). Nematode variables: density, total and ind. biomass, and relative abundances of feeding types 1A (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epistrate feeders) and 2B (predators/scavengers). Station AP3000 represented an outlier for the pigment data and analysis was conducted with and without this station. All analyses, except for POC flux, were run without seamount station GB1200. The number of asterisks denotes the statistical significance level with * $P \leq 0.05$, ** $0.05 < P \leq 0.01$, and *** $0.01 < P \leq 0.001$.*

		$r_{long\|depth}$	$r_{depth\|long}$
POC flux		-0.94***	-0.93***
Chl-a	*incl. AP3000*	-0.54***	-0.24
	excl. AP3000	-0.71***	-0.69*
CPE	*incl. AP3000*	-0.64***	-0.07
	excl. AP3000	-0.77***	-0.53**
Chla:phaeo	*incl. AP3000*	0.2	-0.13
	excl. AP3000	0.31	-0.35
MGS		-0.55***	-0.13
Mud		0.49**	-0.003
TOM		0.42*	0.51**
TOC		0.21	0.14
TN		0.25	0.02
C:N		-0.19	0.09
Density		-0.67***	-0.57***
Total biomass		-0.75***	-0.38*
Ind. biomass		-0.65***	0.09
1A		-0.23	0.05
1B		0.02	0.16
2A		-0.32	-0.22
2B		0.67***	0.1

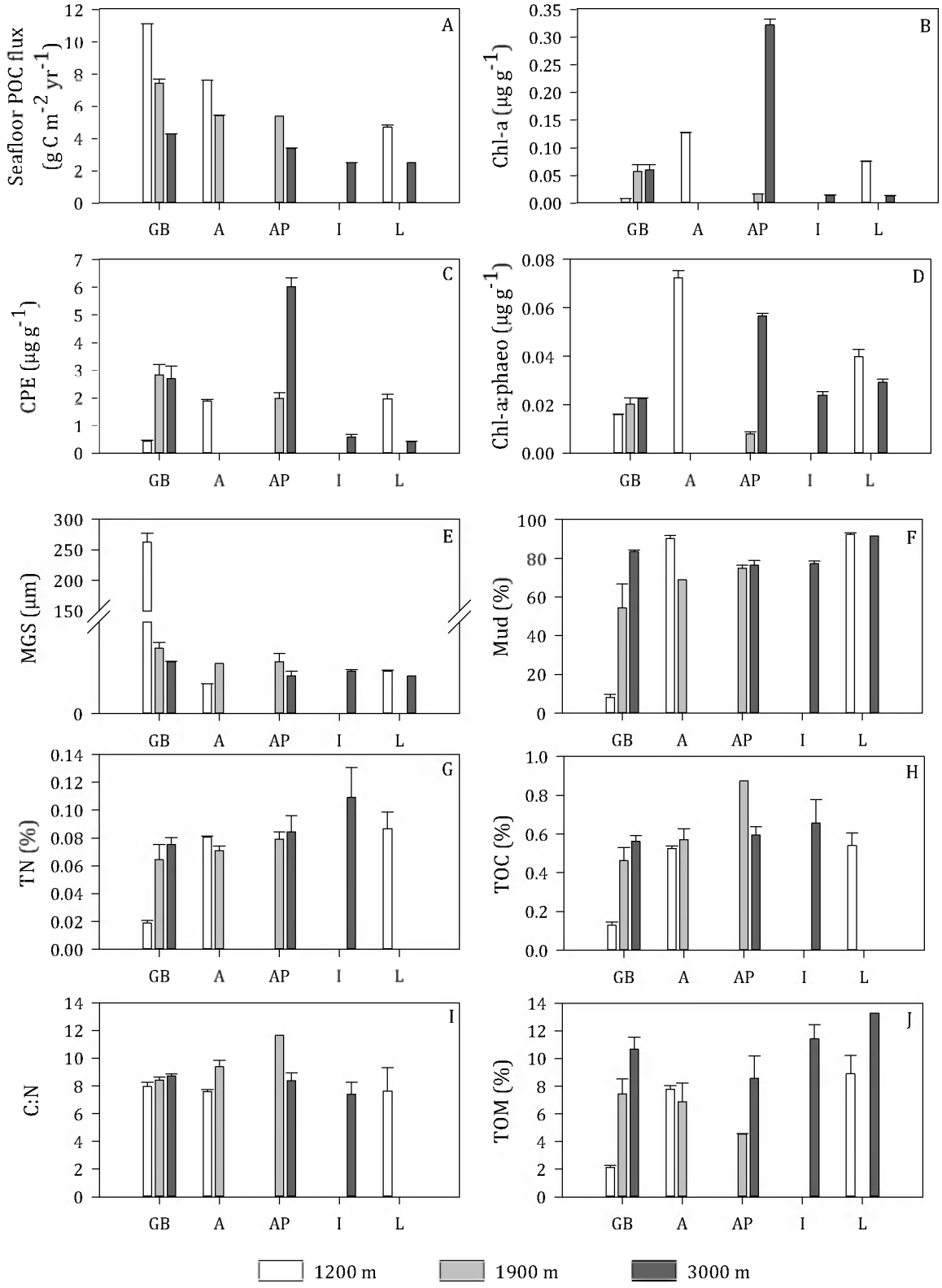

Fig. 3. *Longitudinal and bathymetric trends in environmental variables. The basins sampled are displayed on the x-axis (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin, L: Levantine basin) and are ordered from west to east. The colour of the bars indicates approximate water depth (1200 m: white, 1900 m: grey, 3000 m: dark grey). Bars represent average values; error bars denote standard errors. POC flux: annual particulate organic carbon flux to the seafloor, chl-a: chlorophyll a, CPE: chloroplastic pigment equivalents, chl-a:phaeo: chlorophyll a: phaeopigments ratio, MGS: median grain size, TOM: % of total organic matter, TOC: % of total organic carbon, TN: % of total nitrogen, C:N : molar carbon:nitrogen ratio.*

3.3.2 Longitudinal and bathymetric trends in nematode community characteristics

3.3.2.1 Standing stock

The relative densities of the various meiofaunal taxa encountered at each (seamount + non-seamount) station are listed in the table in the addendum (at the end of this chapter, p 138). Nematodes prevailed at all stations, accounting for 70.0 - 96.1 % of meiofaunal abundance. The second most numerous taxon were the copepods (adults + nauplii; 1.8 – 25.0 %). At some stations polychaetes, rotifers or tardigrades represented more than 1 % of total meiofaunal abundance.

Table 2 contains the coefficients of the Spearman rank correlations for nematode density and biomass with longitude and depth. Nematode standing stock (i.e. total densities and biomass) declined with longitude and with depth. Individual nematode biomass also decreased from west to east but remained constant with increasing water depth.

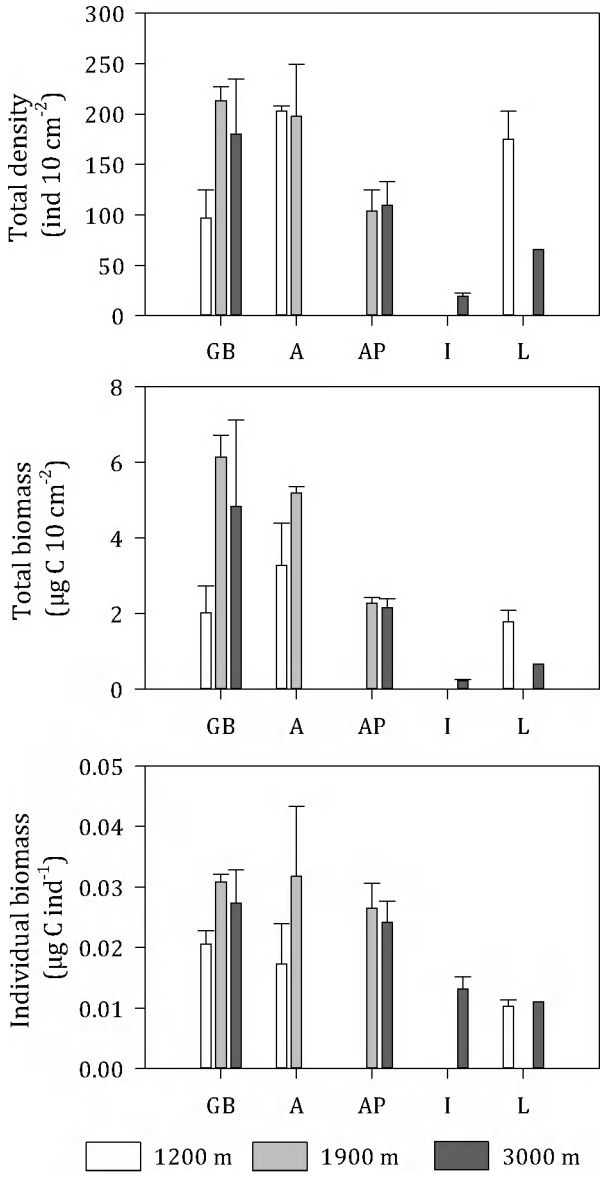

Fig. 4. *Longitudinal and bathymetric trends in nematode densities, total and individual biomass. The basins sampled are displayed on the x-axis (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin, L: Levantine basin) and are ordered from west to east. The colour of the bars indicates approximate water depth (1200 m: white, 1900 m: grey, 3000 m: dark grey). Bars represent average values; error bars denote standard errors.*

3.3.2.2 Genus composition

For all stations (seamount and non-seamount), we recorded 150 nematode genera of which the numerically dominant ones (contributing ≥ 3 % of total abundance) are listed in Table 3. *Acantholaimus* and *Halalaimus* were amongst the dominant genera at every station. Only eleven genera were encountered at all stations. When water depth was fitted first in the DISTLM model (excluding GB1200), there was a graded transition in nematode genus composition from west to

east (sequential tests, depth: P < 0.05; longitude: P < 0.001; Fig. 5). Additionally, when longitude was fixed, depth contributed significantly to the explained variation in genus composition (sequential DISTLM tests, longitude: P < 0.001; depth: P < 0.05). Longitude (depth fitted first: 18.6 %, longitude fitted first: 18.5 %) explained a greater fraction of the variability in nematode genus composition than water depth (depth fitted first: 6.9 %, longitude fitted first: 7.0 %). The BEST analysis showed that at 1200 m depth the genus *Chromadorina* was most responsible (R = 0.99) for the divergence between stations A1200 and L1200, in that it was absent from the latter station. At the 1900 m stations, *Manganonema* and *Spirodesma*, both only found in the GB region, were steering the longitudinal gradient in genus composition (R = 0.62). *Linhomoeus* (absent from the Ionian and Levantine basin), *Metasphaerolaimus* (more prevalent in the Levantine and Ioanian basin) and *Gnomoxyala* (restricted to the Ionian Sea) were the genera mainly responsible for the cline in genus composition at the abyssal stations (R = 0.75).

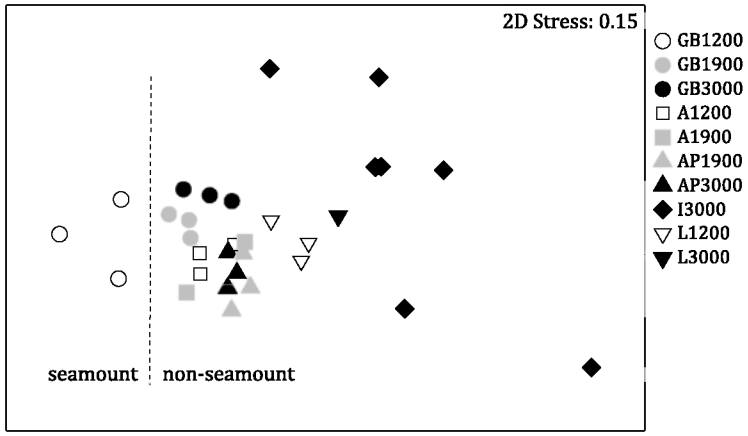

Fig. 5. *Non-metric multi-dimensional scaling (nMDS) plot of fourth-root transformed relative nematode genus abundances per station. The dashed line separates the seamount station from the non-seamount stations. Station codes are explained in Table 1.*

Table 3. *Average (SE) relative abundances (%) of dominant nematode genera (≥ 3 %) per station. The number of replicate samples per station is indicated in Table 1.*

GB1200		**GB1900**		**GB3000**		**A1200**		**A1900**	
Microlaimus/Aponema	12.4 (3.6)	*Acantholaimus*	15.7 (1.5)	*Theristus*	9.6 (1.5)	*Amphimonhystrella*	8.3 (1.6)	*Monhystrella*	11.4 (1.9)
Leptolaimus	11.3 (1.4)	*Daptonema*	14.6 (2.3)	*Acantholaimus*	9.5 (1.5)	*Daptonema*	7.4 (3.3)	*Halalaimus*	7.6 (0.1)
Desmodora	10.3 (4.3)	*Microlaimus/Aponema*	7.9 (3.2)	*Microlaimus/Aponema*	9.5 (1.1)	*Halalaimus*	7.3 (1.0)	*Acantholaimus*	7.3 (4.4)
Bolbolaimus	8.1 (3.5)	*Diplopeltula*	5.0 (2.6)	*Daptonema*	9.1 (1.2)	*Neochromadora*	6.8 (0.9)	*Amphimonhystrella*	7.1 (0.5)
Richtersia	4.2 (2.1)	*Desmoscolex*	4.7 (0.5)	*Tricoma*	7.4 (3.4)	*Acantholaimus*	6.8 (1.2)	*Richtersia*	6.7 (0.9)
Metadesmolaimus	4.0 (1.3)	*Halalaimus*	4.1 (1.3)	*Halalaimus*	6.6 (0.9)	*Leptolaimus*	5.9 (2.7)	*Sabatieria*	6.4 (3.1)
Pselionema	4.0 (1.3)	*Thalassomonhystera*	4.0 (2.1)	*Monhystrella*	6.1 (0.3)	*Pselionema*	4.8 (1.2)	*Daptonema*	5.3 (0.5)
Acantholaimus	3.8 (1.7)	*Leptolaimus*	3.9 (2.7)	*Diplopeltula*	4.0 (0.8)	*Richtersia*	4.2 (2.3)	*Prototricoma*	4.0 (0.7)
Halalaimus	3.8 (0.8)	*Monhystrella*	3.2 (1.2)	*Thalassomonhystera*	4.0 (1.4)	*Desmoscolex*	4.1 (1.2)	*Leptolaimus*	3.7 (2.0)
Daptonema	3.7 (0.5)			*Desmoscolex*	4.0 (0.1)	*Tricoma*	3.7 (2.5)	*Molgolaimus*	3.5 (0.7)
				Prototricoma	3.1 (0.9)	*Monhystrella*	3.5 (0.7)	*Diplopeltula*	3.2 (0.7)

AP1900		**AP3000**		**I3000**		**L1200**		**L3000**	
Acantholaimus	13.5 (2.5)	*Acantholaimus*	13.4 (2.1)	*Monhystrella*	13.2 (5.1)	*Acantholaimus*	10.7 (1.8)	*Acantholaimus*	16.9
Halalaimus	13.5 (0.9)	*Halalaimus*	12.0 (1.6)	*Acantholaimus*	12.7 (3.6)	*Theristus*	5.8 (2.5)	*Halalaimus*	15.4
Neochromadora	5.4 (2.4)	*Amphimonhystrella*	6.7 (0.2)	*Sphaerolaimus*	7.5 (2.9)	*Pselionema*	5.6 (1.8)	*Thalassomonhystera*	10.8
Microlaimus/Aponema	5.4 (2.8)	*Daptonema*	6.6 (2.7)	*Halalaimus*	5.2 (1.5)	*Halalaimus*	5.0 (0.8)	*Metasphaerolaimus*	7.7
Daptonema	4.7 (2.9)	*Monhystrella*	4.4 (0.9)	*Molgolaimus*	5.1 (2.2)	*Richtersia*	4.7 (0.6)	*Enchonema*	4.6
Monhystrella	4.7 (0.2)	*Leptolaimus*	3.5 (0.9)	*Theristus*	5.0 (2.6)	*Sabatieria*	4.7 (1.0)	*Marylinnia*	4.6
Amphimonhystrella	3.8 (1.5)	*Aegialoalaimus*	3.2 (0.7)	*Thalassomonhystera*	4.8 (2.4)	*Molgolaimus*	4.1 (0.3)	*Oxystomina*	4.6
Metasphaerolaimus	3.8 (2.1)			*Metasphaerolaimus*	4.6 (2.5)	*Monhystrella*	3.8 (0.1)	*Pselionema*	4.6
				Aegialoalaimus	3.9 (1.9)	*Marylinnia*	3.8 (0.5)	*Diplopeltula*	3.1
				Sabatieria	3.6 (2.0)	*Prototricoma*	3.8 (0.5)	*Manganonema*	3.1
				Diplopeltula	3.1 (1.7)	*Sphaerolaimus*	3.7 (1.6)	*Sphaerolaimus*	3.1
						Desmoscolex	3.2 (0.8)		

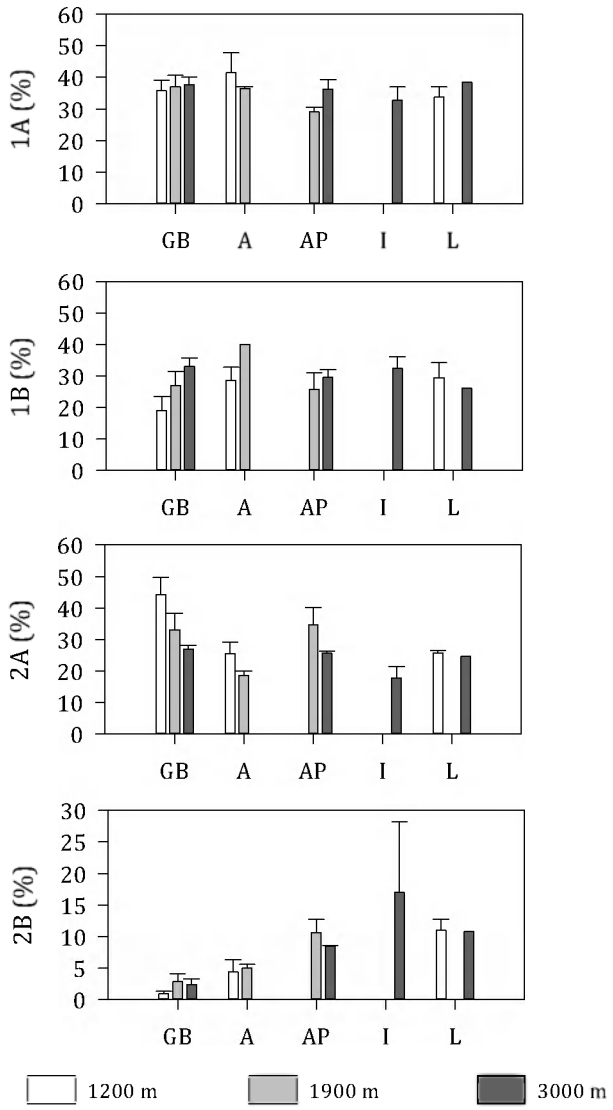

Fig. 6. *Longitudinal and bathymetric trends in the relative abundance of nematode feeding types. The basins sampled are displayed on the x-axis (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin, L: Levantine basin) and are ordered from west to east. The colour of the bars indicates approximate water depth (1200 m: white, 1900 m: grey, 3000 m: dark grey). Bars represent average values; error bars denote standard errors. 1A: selective deposit feeder, 1B: non-selective deposit feeder, 2A: epistrate feeder, 2B: predator/scavenger).*

3.3.2.3 Trophic composition

The trophic structure of the nematode communities at all (seamount + non-seamount) stations is illustrated in Fig. 6. Deposit feeders (1A + 1B) prevailed at all stations and accounted for 45.7 to 85.7 % of total nematode abundance. Predatory/scavenging nematodes (2B) were represented the least (range: 0 - 15.4 %), except for half of the I3000 samples, in which epistrate feeders (2A) attained lowest relative abundances. The relative abundance of predatory/scavenging nematodes displayed a positive longitudinal gradient (Table 2).

3.4 Correlations between seafloor POC flux and benthic environmental variables

Seafloor POC flux showed an inverse relationship with station-averaged TOM (Spearman rank, r = -0.78, P = 0.01).

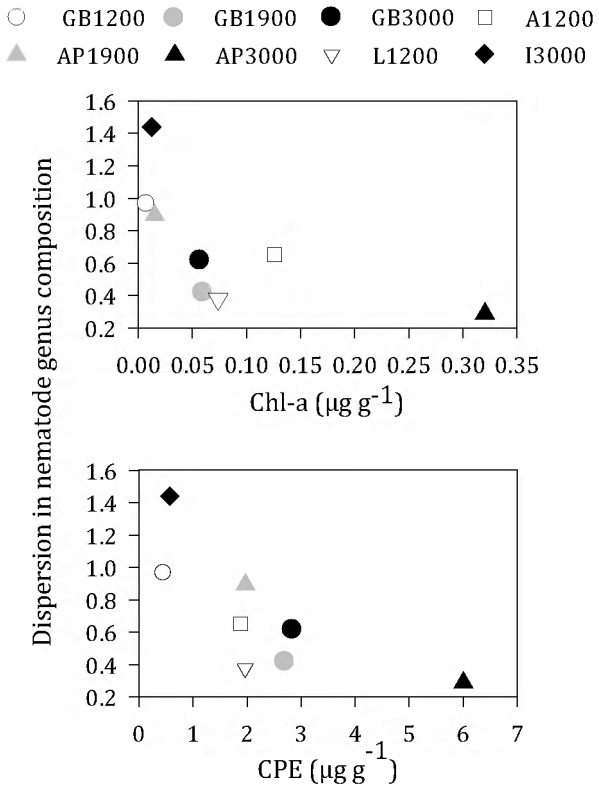

Fig. 7. *Within-station variability in nematode genus composition in function of sedimentary phytopigment concentrations. Top: chlorophyll a (chl-a) concentrations. Bottom: chloroplastic pigment equivalents (CPE). Pigment data were missing for station A1900. Only one sample was collected at L3000, prohibiting the assessment of within-station variability in community structure.*

Table 4. *Spearman rank correlations between station-level nematode community descriptors and environmental variables. Environmental variables: POC flux (annual particulate organic carbon flux to the seafloor), chl-a (chlorophyll a), CPE (chloroplastic pigment equivalents), chl-a:phaeo (chlorophyll a: phaeopigments ratio), TOM (% of total organic matter), TOC (% of total organic carbon), TN (% of total nitrogen), C:N (molar carbon: nitrogen ratio) MGS (median grain size) and mud. Nematode variables: density, total and ind. biomass, and relative abundances of feeding types 1A (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epistrate feeders) and 2B (predators/scavengers). Correlations between genus composition (genus comp.) and environmental variables were obtained through the RELATE procedure. The number of asterisks denotes the statistical significance level with * P ≤ 0.05, ** 0.05 < P ≤ 0.01, and *** 0.01< P ≤ 0.001*

	POC flux	Chl-a	CPE	Chl-a:phaeo	TOM	TOC	TN	C:N	MGS	mud
Density	0.85**	0.62	0.60	0.12	-0.52	-0.88**	-0.71	0.12	0.28	-0.32
Ind. biomass	0.50	0.14	0.69	-0.55	-0.65	-0.10	-0.93**	0.76*	0.63	-0.85**
Total biomass	0.77*	0.40	0.62	-0.26	-0.67	-0.52	-0.98***	0.57	0.57	-0.62
Genus comp.	0.02	-0.17	0.3	-0.23	0.44*	0.40*	0.40*	0.04	-0.12	-0.14
1A	0.20	0.17	-0.14	0.45	0.25	-0.76*	-0.48	-0.10	-0.40	0.27
1B	-0.08	0.24	0.31	0.14	0.15	0.14	0.05	-0.05	-0.02	-0.08
2A	0.35	0.19	0.64	-0.6	-0.45	-0.12	-0.45	0.60	0.65	-0.22
2B	-0.57	-0.38	-0.64	0.17	0.37	0.60	0.79*	-0.36	-0.33	0.38

3.5 Environmental drivers of meiofaunal assemblages

The Spearman rank correlations computed between environmental variables and nematode community descriptors are shown in Table 4. Nematode density and biomass were both correlated with the magnitude of seafloor POC flux, but related more strongly to benthic parameters like TOC (in the case of nematode density) and TN (total biomass). Nematode individual biomass was impacted positively by sedimentary C:N levels, and displayed an inverse relationship with sedimentary mud and TN content. The generic structure of the nematodes related significantly to sedimentary organic matter and nitrogen concentrations. Although chlorophyll pigments were not correlated with nematode genus composition, the variability between replicate samples of the same station declined with increasing pigment concentration (Spearman rank, chl-a: $r = -0.83$, $P < 0.05$; CPE: $r = -0.76$, $P < 0.05$; Fig. 7). After omission of station I3000, from which an aberrantly high number of replicates ($n = 7$) were collected, this relationship was still significant for chl-a (Spearman rank, $r = -0.79$, $P = 0.05$), but not for CPE ($r = -0.68$, $P = 0.11$).

We found only few significant associations between the relative densities of nematode feeding types and environmental variables at the station level (Table 4). Selective deposit-feeding nematodes (feeding type 1A) were affected negatively by sedimentary TOC levels, whereas predatory/scavenging nematodes (2B) contributed more to total nematode abundances in sediments with elevated TN levels.

4 Discussion

4.1 Longitudinal trend in NPP

Our Vertically Generalized Production Modelled (VGPM) NPP data revealed a decline in surface productivity along a longitudinal transect from the GB region to the eastern Mediterranean, in agreement with previously reported *in situ* measurements (Moutin and Raimbault 2002) and other satellite-based estimates (Bosc et al. 2004, D'Ortenzio and Ribera d'Alcalà 2009) for the Mediterranean Sea. The longitudinal drop in productivity was more pronounced for the *in situ* measurements of Moutin and Raimbault (2002) (factor 2.2-2.9) compared to our data (1.8) and those of Bosc et al. (2004) (1.5). Standard algorithms used to convert ocean colour data to chlorophyll concentrations have been proven to systematically overestimate actual concentrations in the Mediterranean, especially in the oligotrophic parts of the basin (Claustre et al. 2002, Bosc et al. 2004). However, the west-east difference in productivity reported in this

study, in which standard algorithms were used, is larger than that recorded by Bosc et al. (2004) who employed a bio-optical algorithm adapted for Mediterranean waters.

The NPP data estimated here were high compared to those reported by previous studies for the same oceanographic regions. This may be related to the usage of different algorithms, which may provide divergent estimates of NPP (Campbell et al. 2002, Carr et al. 2006). Joint el al. (2002) provided a regional NPP estimate for the north-western Iberian margin, where the GB is situated, of ~220 g C m^{-2} yr^{-1}, which is about three times lower than our value for the seamount station (GB1200; 693.5 g C m^{-2} yr^{-1}). This may point to a local maximum in NPP associated with the GB, which has been observed before for some seamounts (Clark et al. 2010). However, unlike seamount station GB1200, the deeper stations in the GB region experience, besides a chlorophyll maximum in spring, a NPP peak in late summer, resulting in higher annual NPP. This second NPP maximum may result from offshore transport of phytoplankton produced at the shelf following coastal upwelling during summer (Joint et al. 2002). Also for the various Mediterranean regions, our NPP approximations (Algerian basin: 688.7 g C m^{-2} yr^{-1}, Algero-Provençal basin: 556.2 ± 9.3 g C m^{-2} yr^{-1}, Ionian basin: 400.0 g C m^{-2} yr^{-1}, Levantine basin: 384.1 g C m^{-2} yr^{-1}) were consistently a factor 3 to 4 higher in comparison with the satellite-based values of Bosc et al. (2004) (Algerian basin: 162.5 g C m^{-2} yr^{-1}, Algero-Provençal basin: 153 g C m^{-2} yr^{-1}, Ionian basin: 120.4 g C m^{-2} yr^{-1}, Levantine basin: 106.3 g C m^{-2} yr^{-1}). Because of the presence of Saharan dust in Mediterranean surface waters, standard algorithms, such as VGPM, tend to overestimate NPP in this basin (Claustre et al. 2002). Bosc et al. (2004) employed a regional algorithm, adapted for Mediterranean waters, which was believed to generate less biased NPP values.

4.2 Longitudinal trend in seafloor POC flux

The productivity gradient between the GB region and the eastern Mediterranean was accompanied by a reduction in seafloor POC flux, conforming with sediment trap data for the Mediterranean basin (Danovaro et al. 1999a). However, several factors may have resulted in a bias in these estimated POC flux data.

The POC flux calculated for the seamount station was most probably an overestimate since local strong near-bottom currents may have diverted the POC rain (Smith et al. 2001, Gorsky et al. 2002, 2003). The coarse sediment texture at the GB seamount was previously ascribed to the winnowing of the fine sediment fraction by strong bottom currents (van Weering et al. 2002, Duineveld et al. 2004). At seamount station GB1200, maximal estimated POC deposition was associated with minimal benthic pigment concentrations, pointing to a decoupling between NPP

and POC deposition (but see 4.3). However, the POC flux we approximated for GB1200 (Fig. 2A) was similar to the values of Duineveld et al. (2004) at 800 m depth (6.2 – 12.8 g C m^{-2} yr^{-1}).

The POC sedimentation rates estimated for the Mediterranean stations were either lower than or in the same range as those given by previous research. At 3000 m depth in the Algero-Provençal basin, Zúñiga et al. (2007) recorded a POC flux of 1 g C m^{-2} yr^{-1}, which is one third of the value we found for station AP3000. In the Antikythira strait, which connects the Aegean with the Ionian Sea, the amount of POC collected with a sediment trap placed 1345 m deep was 3.9 g C m^{-2} yr^{-1} (Kerhervé et al. 1999), which is of the same order as the POC flux we estimated for the same depth in the Levantine Sea (L1200: 4.7 ± 0.1 g C m^{-2} yr^{-1}). In the Cretan Sea, near northern Crete, Danovaro et al. (1999a) measured a four times lower POC flux of 1.2 g C m^{-2} yr^{-1}, inferred from sediment trap data. The divergence between modelled POC flux and that measured by sediment traps may be related to the systematic bias associated with the latter related to, amongst others, the inclusion of swimmers, hydrodynamic activity, and degradation of trapped organic material (Gardner 2000).

Additional potential bias in the POC flux estimated via the algorithm of Lutz et al. (2007) may be introduced by (1) the limited data coverage of the algorithm relative to the entire ocean surface (incl. only two (rather productive) locations from the Mediterranean), and (2) the fact that the seasonal variability in NPP is not the only factor that governs the amount of POC that is transported to depth. First of all, POC that sinks from the euphotic zone to depth does not only comprise phytoplankton-derived material, but also consists of faecal pellets and moults of zooplankton (Gage 2003, De La Rocha and Passow 2007). The contribution of faecal pellets to the POC rain can be substantial (up to 100 % of the total POC flux) and varies between regions (Ducklow et al. 2001). Secondly, the composition and activity (which is affected by seawater temperature) of pelagic zooplankton and bacterial communities, as well as the overall food-web structure determines the efficiency of the biological pump (Legendre and Rivkin 2002). Siokou-Frangou et al. (2010) describe the disparity in the composition of planktonic communities between the western and the eastern Mediterranean, which may lead to differences in POC transport efficiency. The inclusion of data on circulation patterns, and the community and food web structure of the plankton may lead to a refinement of the algorithm to estimate seafloor POC flux.

4.3 Benthic-pelagic coupling: effects on the seafloor environment

The longitudinal cline in surface productivity and resulting sedimentation rates between the GB region and the Levantine basin was mirrored in the benthic phytopigment concentrations, consistent with Pusceddu et al. (2010) and Gambi and Danovaro (2006).

At seamount station GB1200 (high POC flux, low pigment concentrations) and abyssal plain station AP3000 (low POC flux, high pigment concentrations), seafloor POC flux data did not appear to match with sedimentary pigment levels. We argue that the high pigment levels observed for AP3000 relative to the GB stations and A1200 are primarily caused by the different timing of the expeditions during which these stations were sampled. The pigment samples from AP3000 were collected in June, when NPP values were nearly maximal (Fig. 2B). In contrast, sampling in the GB region and at station AP1900 was conducted in October-November when NPP was at a low. In June, however, NPP values for the GB region exceeded those for the western Mediterranean. Thus, at least part of the divergence between seafloor POC flux and pigment concentrations can be attributed to the usage of annual composite POC flux data, which do not take into account seasonal heterogeneity. However, the difference in pigment concentrations between A1200 and AP3000, both sampled in June, cannot be attributed to seasonal sampling differences. The smaller pigment pool at A1200 may be the consequence of the more extensive, total (i.e. mega-, macro-, and meiofauna, as well as prokaryotes) benthic community at this shallower depth (Rex et al. 2006, Wei et al. 2010). As for the seamount station, the discrepancy between POC deposition and pigment concentrations may also be a result of the usage of annual composite POC flux in combination with pigment data obtained in October. Nonetheless, an additional explanation for the low pigment concentrations encountered at GB1200 is the strong hydrodynamic forcing, sweeping away fine phytodetrital matter (see 4.1).

In contrast to Relexans et al. (1996), Gambi and Danovaro (2006) and Lampadariou et al. (2009), we did not observe lower sediment TOC, TN or TOM values at the less productive stations. Bulk concentrations of these elements do not necessarily represent the amount of available food, since part of nitrogen or carbon containing material might be either intrinsically refractory or unavailable for consumption owing to physical protection by organic or inorganic matrices (Burdige 2007). Hence, we consider TOC, TN, TOM and C:N to be potential but not definite nematode food indicators. In addition, although these variables are often regarded as a measure of POC deposition, they can be more closely associated with grain size (owing to its relationship with surface: volume ratios) than with OM delivery (Mayer 1994, Hedges and Keil 1995). In the present study, however, we did not find statistically significant relations between sediment grain size and TN, TOC or TOM.

4.4 Benthic-pelagic coupling: effects on nematode standing stock

The present study supports the general notion that deep-sea sediments underlying productive waters harbour elevated meiofaunal (nematode) standing stock (Relexans et al. 1996, Galeron et al. 2000, Gambi and Danovaro 2006, Lampadariou and Tselepides 2006, Lampadariou et al. 2009, Bianchelli et al. 2010, Gambi et al. 2010). Also the deep-sea megabenthos attains higher biomass in the more productive western Mediterranean compared to the more oligotrophic eastern basin (Tecchio et al. 2011).

The correlation analysis pointed towards the importance of food availability, in terms of POC sedimentation rates and even more importantly sedimentary TN and TOC levels, for nematode standing stock. However, the results obtained for the seamount station indicate that food may not be the only determinant of nematode biomass. Correspondingly, in his review on meiofauna along continental margins, Soltwedel (2000) noticed considerable variation in the relationship between CPE and meiofaunal abundances between geographic regions, which he attributed to the interference of other environmental and/or biotic factors. The unusually low standing stock at the seamount was believed to be the result of the strong hydrodynamic forcing (inferred from the coarse and well-sorted sediments; Van Weering et al. 2002, Duineveld et al. 2004) either through the exertion of physical stress or through the low food availability that comes with it, or both. Food shortage at seamount station GB1200 is suggested by the low amounts of phytopigments, nitrogen and organic matter within the sediments. In support, Thistle and Levin (1998) documented reduced nematode abundances under experimental strong near-bottom flow.

Opposed to Danovaro et al. (2008b), the longitudinal productivity gradient was accompanied by a significant drop in individual nematode biomass. Based on an extensive literature analysis, Udalov et al. (2005) described a positive effect of grain size and food availability on individual nematode biomass. In contrast, Soetaert et al. (2009), who analyzed raw biomass data, found no effect of grain size on individual biomass. In our study area, nematode individual biomass decreased with increasing sedimentary mud content, but strangely also with sedimentary TN concentrations. This finding suggests that bulk TN concentrations might not represent a suitable proxy for the amount of food available to nematodes. Note that the lack of a relationship between nematode biomass and labile phytodetritus, a potentially better measure for food availability than TN, may be the result of the fact that we had to average environmental and faunal variables per station, thereby expunging (co)variation in both parameters at a smaller spatial scale.

4.5 Benthic-pelagic coupling: effects on nematode community structure

There was a highly significant and strong reduction in core surface area from the GB region to the eastern Mediterranean (Spearman rank, $r = -0.74$, $P < 0.001$). Thus, since sample volume might have an impact on genus composition (smaller samples might contain comparatively less rare genera than larger sediment samples), the observed longitudinal gradients could result partly from the heterogeneity in core surface area. Nevertheless, the generic composition of nematode assemblages changed gradually from the GB region to the eastern Mediterranean basin together with several benthic environmental variables (TN, TOC and TOM). The significance of (potential) food availability to the structure of nematode assemblages was also demonstrated by Ingels et al. (2011b) (TN, chl-a, chl-a:TOC) and Fonseca and Soltwedel (2009) (particulate proteins and phospholipids). Similar to Fonseca and Soltwedel (2009), who studied nematode species composition in the Arctic, we noted increased variability in nematode community structure among replicate samples in sediments with reduced phytopigment concentrations. Fonseca and Soltwedel (2009) invoked the energy-richness hypothesis (Hawkins et al. 2003) as an explanation for this pattern. According to this hypothesis, low energy levels result in small population sizes of species, and local stochastic extinction events restrict species' distribution ranges.

The gradual change in nematode trophic structure from west to east was mainly driven by the increased relative abundance of predatory/scavenging nematodes (mainly *Sphaerolaimus* and *Metasphaerolaimus*) with longitude. Although they found no statistically significant relationship between predator/scavenger abundance and longitude, Danovaro et al. (2008b) also noticed a higher representation of this feeding guild in the eastern compared to the western Mediterranean. The lower fraction of predatory/scavenging nematodes in the more productive western part of our transect implies that members of this feeding guild do not relate directly to the supply of surface-derived OM. As Gage (2003) stated, in oligotrophic regions, organisms feeding upon sedimented POC may suffer a disadvantage compared to those that do not. In support, Sibuet et al. (1993) counted most necrophagous amphipods at the most oligotrophic site in the tropical Atlantic. In contrast, sediments from the Nazaré canyon (Ingels et al. 2009) and from several Mediterranean canyons (Soetaert and Heip 1995), which receive high POC loadings, harboured a higher percentage of predators/scavengers relative to adjacent open slope stations. This paradox calls for more detailed investigations into the environmental drivers of predator/scavenger abundances.

Epistrate feeders were especially abundant at the GB seamount, similar to Maud Rise in the Antarctic (Guilini et al. 2013) and in sediments surrounding the Paluniro seamount in the western Mediterranean (Pusceddu et al. 2009). However, at the Marsili seamount in the western

Mediterranean the share of epistrate feeders was limited and as such the dominance of this feeding type in seamount sediments cannot be generalized. Nevertheless, there were very few seamount studies addressing nematode community structure with which we could compare our results, and much more research in this field is definitely needed.

4.6 Conclusions

Along the longitudinal transect from the Galicia Bank region to the eastern Mediterranean, downward benthic-pelagic coupling was evident in terms of phytopigment concentrations, and in standing stock, individual biomass, genus and trophic composition of nematodes in bathyal and abyssal sediments. Standing stock seemed to be regulated by POC deposition and benthic potential food indicators (i.e. percentage of nitrogen, organic carbon, and total organic matter), whereas genus composition was only related to the latter. The significant relationship between nematode parameters and POC flux does not necessarily imply these organisms feed upon the sedimented OM directly; for instance, bacteria, another potential nematode food source, are often associated with phytodetritus (Danovaro et al. 1999b).

Climate change is expected to modify the biogeochemical fluxes to the deep sea, which regulate the community structure and function of deep-sea benthic communities (Smith et al. 2008). Long-term studies in the northeast Pacific and at the Porcupine abyssal plain have revealed climate-driven variation in the community structure of foraminiferans, mega- and macrofauna in abyssal sediments (Smith et al. 2009). The coupling between bathyal and abyssal nematode assemblages and surface water processes as evidenced in the present study suggests that it is likely that climate change will affect the composition and function of deep-sea nematodes as well.

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meiofauna and preparation of nematode slides by Annick Van Kenhove and Guy De Smet were greatly appreciated.

Table 1. *Average (SE) relative meiofaunal taxon densities (%) per station (0-1 cm sediment depth).*

	GB1200	GB1900	GB3000	A1200	A1900	AP1900	AP3000	I3000	L1200	L3000
Amphipoda	0.08 (0.08)	-	-	-	-	-	-	-	-	-
Copepoda										
adults	2.98 (1.49)	3.11 (0.44)	3.69 (0.49)	3.36 (0.51)	2.20 (0.39)	6.03 (0.91)	4.79 (0.14)	6.43 (2.55)	2.95 (1.48)	1.37
nauplii	4.32 (1.04)	3.35 (0.38)	5.12 (0.11)	3.74 (0.62)	2.85 (1.20)	4.49 (1.16)	5.82 (0.63)	6.44 (1.80)	3.53 (1.88)	4.11
Cumacea	-	-	0.03 (0.03)	-	-	-	-	-	-	-
Gnathostimulida	-	0.02 (0.02)	-	-	-	-	-	-	-	-
Halacaroidea	-	0.04 (0.04)	-	-	-	-	0.06 (0.06)	-	-	-
Holothuroidea	-	-	-	-	-	-	-	-	0.60 (0.60)	-
Isopoda	0.04 (0.04)	0.07 (0.07)	0.27 (0.21)	-	-	-	-	-	-	-
Kinorhyncha	-	0.18 (0.10)	0.18 (0.06)	0.29 (0.14)	0.04 (0.04)	0.29 (0.04)	-	0.71 (0.71)	0.69 (0.43)	-
Nematoda	90.69 (2.67)	91.09 (0.72)	89.86 (0.76)	91.08 (1.26)	91.13 (3.87)	86.81 (1.57)	87.96 (0.65)	83.85 (3.18)	89.72 (1.95)	91.78
Oligochaeta	-	0.02 (0.02)	-	-	-	-	-	-	-	-
Ostracoda	0.21 (0.11)	0.19 (0.08)	0.23 (0.09)	-	0.04 (0.04)	-	-	-	-	-
Polychaeta	1.02 (0.24)	1.03 (0.07)	0.60 (0.19)	0.15 (0.02)	0.39 (0.05)	1.12 (0.26)	0.46 (0.16)	-	1.55 (0.56)	1.37
Rotifera	0.15 (0.15)	0.23 (0.08)	0.03 (0.03)	0.49 (0.28)	2.39 (2.34)	0.12 (0.06)	0.19 (0.01)	1.04 (0.68)	0.47 (0.28)	-
Tanaidacea	0.03 (0.03)	0.04 (0.02)	-	-	-	-	-	-	-	-
Tardigrada	0.48 (0.12)	0.64 (0.13)	-	0.88 (0.17)	0.95 (0.09)	1.31 (0.38)	0.71 (0.05)	1.53 (1.14)	0.16 (0.16)	1.37
Turbellaria	-	-	-	-	-	-	-	-	0.33 (0.16)	-


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Chapter 5

Unravelling the environmental drivers of deep-sea nematode biodiversity and its relation with carbon mineralisation along a longitudinal primary productivity gradient

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ABSTRACT - Alongside a primary productivity gradient between the Galicia Bank region in the northeast Atlantic and the more oligotrophic eastern Mediterranean basin, we investigated the bathymetric (1200 - 3000 m) and longitudinal variation in several measures for nematode taxon (Shannon-Wiener genus diversity, expected genus richness and generic evenness) and functional diversity (trophic diversity, diversity of life history strategies, biomass diversity and phylogenetic diversity). Our goals were to establish the form of the relation between diversity and productivity (measured as seafloor particulate organic carbon or POC flux), and to verify the positive and negative effect of sediment particle size diversity (SED) and the seasonality in POC flux (SVI), respectively, on diversity, as observed for other oceanographic regions and taxa. In addition, we hypothesized that higher taxon diversity is associated with higher functional diversity, which in turn stimulates nematode carbon mineralisation rates (determined from biomass-dependent respiration estimates). Taxon diversity related positively to seafloor POC flux. Phylogenetic diversity (measured as average taxonomic distinctness) was affected negatively by the magnitude and variability in POC flux, and positively by SED. The latter also showed an inverse relation with trophic diversity. Accounting for differences in total biomass between samples, we observed a positive linear relation between taxon diversity and carbon mineralisation in nematode communities. We could, however, not identify the potential mechanism through which taxon diversity may promote this ecosystem function, since none of

the functional diversity indices related to both diversity and nematode respiration. The present results suggest potential repercussions of climate change on deep-sea ecosystem functioning, but further also emphasize the need for a better understanding of nematode functions and their response to evolutionary processes.

1 Introduction

Biodiversity within deep-sea sediments exhibits clear geographic variation. Potentially simultaneously acting drivers of variation in local diversity include productivity, boundary constraints, sediment heterogeneity, oxygen availability, hydrodynamic regimes and catastrophic physical disturbance (Levin et al. 2001). Gradients in these environmental factors co-determine local diversity by influencing the rates of local processes like resource partitioning, competition, predation, physical disturbance etc. Bathymetric variation in diversity is one of the most studied geographical diversity trends (e.g. Danovaro et al. 2008b, Rex and Etter 2010, Tecchio et al. 2011). Benthic diversity generally shows a hump-shaped bathymetric pattern, with a peak around 1500-2500 m depth (Stuart et al. 2003, Rex and Etter 2010). However, the unimodal relationship between diversity and water depth is not universal and the form of the association varies between regions (Stuart et al. 2003, Danovaro et al. 2010). The depth-related gradient in diversity is believed to be governed by productivity (i.e. the particulate organic carbon (POC) flux) and/or sediment characteristics (Gray 2002, Stuart et al. 2003). Deep-sea diversity has been documented to vary positively (Lambshead et al. 2000, 2002, Glover et al. 2002), negatively (Gooday et al. 2012) or unimodally (Tittensor et al. 2011, Leduc et al. 2012b, McClain et al. 2012) with productivity for different taxa and geographic regions. The magnitude of productivity is assumed to have a positive effect on diversity (through the stimulation of population growth), whilst temporal variability in productivity may depress diversity (by limiting feeding to certain periods of the year) (Chown and Gaston 1999, in Rex and Etter 2010). Seasonality in surface productivity had an adverse effect on foraminiferal species diversity in abyssal sediments (Corliss et al. 2009, Gooday et al. 2012). The diversity of sediment particles, which can be regarded as a measure of habitat heterogeneity, has a positive influence on macrofaunal (Etter and Grassle 1992) and nematode (Leduc et al. 2011) species diversity in the western North Atlantic and in the Pacific Ocean, respectively.

As a consequence of the worldwide ongoing decline in marine and terrestrial biodiversity (Pereira et al. 2010) there has been an explosion in the number of studies addressing the effect of biodiversity on the functioning of ecosystems (reviewed by Hooper et al. 2002, Balvanera et

al. 2006, Stachowicz et al. 2007). There are four main possible impact scenarios of biodiversity on an ecosystem function: (1) no effect (null model); (2) all taxa (species/genera...) contribute to ecosystem functioning (rivet hypothesis), (3) there is a minimum need of species, and all other species are redundant (redundancy model); (4) the effect is not predictable (idiosyncratic model) (Lawton 1994, Naeem et al. 1995). According to different authors, the nature and strength of the relation between diversity and an ecosystem function depends on the environmental factors that drive diversity and ecosystem processes (Cardinale et al. 2000, Bengtsson et al. 2002) and the ecosystem function considered (Naeem et al. 1995, Bolam et al. 2002, Hiddink et al. 2009).

Numerous biodiversity-ecosystem function studies related taxon diversity (i.e. the diversity of taxa, with taxa indicating species, genera or other taxonomic levels), and primarily taxon richness (i.e. the number of taxa), to the rate of ecosystem processes, assuming this diversity measure serves as an adequate surrogate for functional diversity (Naeem and Wright 2003). However, taxa may differ in their contribution to total functional diversity (degree of redundancy and singularity) and/or total abundance (commonness-rarity), resulting in a huge variety in possible relationships between taxon and functional diversity (Naeem and Wright 2003, Cadotte et al. 2011). Moreover, the nature of the relation between taxon and functional diversity depends on the measure of functional diversity employed (Naeem and Wright 2003). Analogous to taxon diversity, different aspects of functional diversity can be measured, namely richness, divergence and evenness (Mason et al. 2005). Numerous univariate and multivariate indices have been developed that fall into one of these categories (Weiher 2011). Because functional diversity provides a direct mechanistic link between diversity and ecosystem functioning, a growing amount of research has been devoted to the effect of functional - instead of taxon - diversity on ecosystem functioning (Díaz and Cabido 2001, Petchey et al. 2004, Reiss et al. 2009). In many studies where both functional and taxon diversity were related to the rate of ecosystem processes, functional diversity or composition explained a greater portion of ecosystem functioning than traditional measures of taxon diversity (Díaz and Cabido 2001, Petchey et al. 2004).

Contrary to taxon diversity, phylogenetic diversity entails the evolutionary relationships amongst taxa (Vellend et al. 2010). When it is difficult to identify or measure those properties that are relevant to the ecosystem function under study, phylogenetic diversity may be a useful proxy for functional diversity, since it often encompasses most of the variation in functional traits within a community (Cadotte et al. 2011, Srivastava et al. 2012). The rationale behind this approach is that phylogenetic relatedness usually indicates ecological resemblance, i.e. the more closely related two individuals are, the higher the likelihood that they are functionally similar

(but see e.g. Srivastava et al. 2012, Gravel et al. 2012). Cadotte et al. (2008, 2009) discovered that phylogenetic diversity was a better predictor of ecosystem functioning than both species and functional group richness. Moreover, not only individuals belonging to different species may differ in functional characteristics, also considerable intraspecific variability in functional traits is known to occur (Messier et al. 2010, Bolnick et al. 2011). This finding calls for a trait-based instead of a taxon-based approach in examining the effect of diversity on ecosystem functioning.

Deep-sea nematodes are highly diverse (Lambshead and Boucher 2003), and owing to their omnipresence they can be used to study broad-scale geographic patterns in diversity (Lambshead et al. 2002) as well as the importance of diversity to ecosystem functioning (Danovaro et al. 2008a). Here, we investigated the variation in nematode taxon and functional diversity along longitudinal (reaching from the Galicia Bank in the northeast Atlantic to the eastern Mediterranean basin) and bathymetric (1200 – 1900 – 3000m) gradients within deep-sea sediments. The first aim of this study was to identify potential environmental drivers (i.e. magnitude and variability in seafloor particulate organic carbon (POC) flux and sediment particle size diversity) of nematode taxon and functional diversity. Specifically, we explored the form of the diversity-productivity curve (unimodal, positive or negative) by characterizing the relationship between nematode diversity and the magnitude of the POC flux to the seabed. Our second aim was to determine how nematode diversity relates to ecosystem functioning. Concretely, we presumed that higher taxon diversity results in higher functional diversity, which in turn stimulates nematode carbon mineralisation. The rate of carbon mineralisation by the nematode community was assessed by estimating respiration rates from biomass measurements.

2 Materials and methods

2.1 Study region and sampling strategy

Sediment samples were collected at 1200, 1900 and 3000 m water depth along a longitudinal transect spanning the Galicia Bank in the northeast Atlantic and the Mediterranean basin (Fig. 1, Table 1). The regions that were sampled were, from west to east, the Galicia Bank region, and the Algerian, Algero-Provençal, Ionian and Levantine basin in the Mediterranean Sea. Samples comprised either sub-samples from box cores taken with multicorer cores or actual multicorer samples. We used cores with differing surface areas (see 2.3), but standardized subsamples of maximum 100 nematodes per sediment layer were used for diversity analysis. Sediment cores

were sliced horizontally per cm down to 5 cm, and from 5 to 10 cm sediment depth. Next, these sediment sections were fixed in seawater-buffered 4 % formalin.

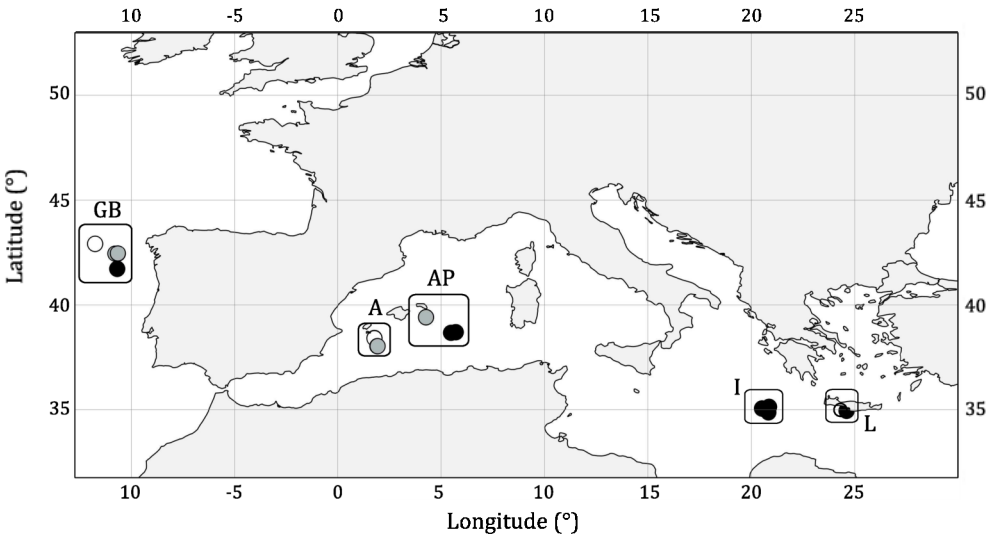

Fig. 1. *Map with sampling locations. Colours indicate approximate water depth (white: 1200 m, grey: 1900 m, black: 3000 m). The rectangles enclose samples that were collected within the same region (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin and L: Levantine basin).*

Table 1*. Sampling details. Indicated are the region where samples were collected in (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin, I: Ionian basin and L: Levantine basin), station code (representing region and approximate water depth), latitude and longitude (in decimal degrees; where rounded coordinates of replicates differed, a range is given), range of water depths over replicates, number of replicate samples, surface area of the core, and the research vessel (RV) aboard which samples were taken (SDG: Sarmiento de Gamboa).*

Region	Period	Station	Lat	Long	Depth (m)	Nr of replicates	Core area (cm^2)	RV
GB	Jun/2008	GB1200	42.9	-11.8	1139-1141	3	78.54	*Belgica*
GB	Oct/2008	GB1900	42.4-42.5	-10.7	1770-1896	3	70.88	*Pelagia*
GB	Oct/2008	GB3000	41.7	-10.7	3066-3072	3	70.88	*Pelagia*
A	Jun/2009	A1200	38.4	1.8	1211-1214	3	69.40	*SDG*
A	Jun/2009	A1900	38.0	1.9	2004, 2016	2	69.40	*SDG*
AP	Nov/2009	AP1900	39.4	4.3	1582	3	56.45	*Pelagia*
AP	Jun/2009	AP3000	38.7	5.5-5.7	2841-2846	3	69.40	*SDG*
I	Jun/2008	I3000	34.9-35.1	20.5-20.8	2770-2807	7	10.18	*Urania*
L	Jun/2008	L1200	35.0	24.6	1026-1143	3	10.18	*Urania*
L	Jun/2008	L3000	34.9	24.5	2647	1	10.18	*Urania*

2.2 Environmental variables

Granulometric data were available for the top 5 cm of the sediment, and were averaged over the five sediment depth layers. Sediment particle size diversity (SED) was computed as the Shannon-Wiener diversity index based on the percent dry weight of 10 particle size classes (i.e. <4, 4-38, 38-63, 63-125, 125-250, 250-500, 500-800, 800-1000, 1000-1600, >1600 µm) (Etter and Grassle 1992, Leduc et al. 2011). The seasonal variability in surface primary productivity (SVI) was calculated as the coefficient of variation (i.e. standard deviation divided by the mean) of monthly NPP values (Lutz et al. 2007), which were extracted from the Vertically Generalised Production Model (VGPM; resolution: 1°) (Behrenfeld and Falkowski 1997) and downloaded from http://www.science.oregonstate.edu/ocean.productivity/. We considered SVI as a proxy for the intermittency with which organic matter is deposited at the deep-sea bed (referred to as seasonality or seasonal variability in POC flux in the remainder of the text). The VGPM estimate of NPP values was based on satellite measurements of sea surface temperature (SST), surface water chl-a concentrations, and photosynthetically active radiation. Data on the particulate organic carbon (POC) flux to the seafloor (abbreviated as POC in the remainder of the text) were approximated on the basis of water depth and SVI values following Lutz et al. (2007).

2.3 Nematode diversity

The formalin-fixed sediment samples were washed over a 32 µm mesh sieve and the meiofauna extracted from the sediment by Ludox centrifugation (Heip et al. 1985). Where possible, around 100 nematodes were hand-picked from each sediment layer and identified to genus level. Genus abundance data for the top 0 to 10 cm of each sediment core were obtained by summing genus counts in all sediment slices, taking into account total nematode abundances in each slice. Diversity indices were calculated per core and hence signify point diversity values. Genus diversity was evaluated by means of expected genus richness EG(20), Pielou's evenness (J'), as well as Shannon-Wiener diversity (H', $\log_e$), which incorporates both the number of genera and their relative abundances. Functional nematode diversity was assessed using the following metrics:

- On the basis of the morphology of the buccal cavity, nematode genera can be appointed to one of the following four feeding types: selective deposit feeder (1A), non-selective deposit feeder (1B), epistrate feeder (2A) and predators/scavengers (2B) (Wieser 1953). Nematode *trophic diversity* (TD) was computed as the reciprocal of the trophic diversity index given by Heip et al. (1985):

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

with q_i = the relative abundance of feeding type i. Consequently, the value of TD varied between 1 (all individuals belong to the same feeding guild) and 4 (all 4 feeding types comprise the same number of individuals). Since all four feeding guilds were represented in all sediment cores studied, TD could be considered as a measure of trophic evenness (Mason et al. 2005).

- Based on their life history strategies, nematode genera can be assigned a c-p score ranging between 1 (colonizers: short generation time, high reproduction rate and colonization ability and tolerant towards pollution and disturbance) and 5 (persisters: long life cycle, low reproduction potential, sensitive to disturbance and pollution) (Bongers 1990). Genera with a c-p score of 2, 3 or 4 are intermediate between colonizers and persisters. Monhysterid genera were assigned to the c-p 2 class ("general opportunists") as advised by Bongers et al. (1995), and as such there were no nematodes belonging to c-p class 1 ("enrichment opportunists"). We calculated the Shannon-Wiener diversity index based on the partitioning of nematode individuals over the 4 c-p classes encountered, and termed this *c-p diversity*.

- As measures for taxonomic or phylogenetic diversity (not to be confused with the "phylogenetic diversity index" PD which is an example of a phylogenetic diversity index, see Clarke and Warwick 2001a), we calculated *average taxonomic distinctness* based on quantitative (Δ^*) and presence-absence data (Δ^+) (with lower distinctness indicative of a higher average relatedness), as well as the *variation in taxonomic distinctness* (Λ^+, a measure for the imbalance of the taxonomic tree, based on presence-absences) (for formulas see Warwick and Clarke 1998, Clarke and Warwick 2001b, Clarke and Gorley 2006). Assuming that Λ^+ indicates functional unevenness, and higher values point to less functionally diverse communities, we used $1/\Lambda^+$ to quantify taxonomic or functional evenness. The two average taxonomic distinctness metrics measure functional divergence. Using the ellipse plots in the TAXDEST routine in Primer, we investigated whether Δ^+ and Λ^+ were mechanistically related (Clarke and Warwick 2001b). We used the following taxonomic levels to calculate the phylogenetic or taxonomic diversity indices: class, subclass, order, suborder, superfamily, family and genus, according to the classification by De Ley et al. (2006), and assumed equal step length.

- Finally, we measured length (L, µm) and width (W, µm) of all nematodes that were mounted on slides for identification purposes to estimate individual wet weight (WW) using Andrassy's (1956) formula, adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm^{-3}; µg WW = L x W^2/1 500 000). Individual biomass (B) in terms of µg C ind^{-1} was then calculated as 12.4 % of WW (Jensen 1984). Next, we calculated *biomass diversity* (BD) using a Shannon-Wiener diversity expression adapted for continuous variables according to Quintana et al. (2008). The computation was performed in the Diversity08 software available at http://limnolam.org/.

Taxon (genus) and phylogenetic diversity indices were calculated in Primer v6 (Clarke and Gorley 2006).

In addition to these diversity indices, we computed the *Maturity index* (MI) of a nematode assemblage as the weighted average of the individual genus c-p values:

$$MI = \sum_{i=1}^{n} v(i)f(i)$$

with v(i) = the c-p value of genus i and f(i) the relative abundance of that genus (Bongers 1990, Bongers et al. 1991, 1995). Hence, the higher the relative abundance of nematode genera with a high c-p score, the higher the value of MI. This functional response measure gives an idea about how stable is the environment in which nematodes live.

2.4 Nematode respiration

Individual nematode respiration rates (R; µg C ind^{-1} d^{-1}) were calculated on the basis of individual biomass (B) using the formula of de Bovée and Labat (1993):

$$R = 0.0449 \times B^{0.8554} \times exp^{lnQ10/10(T-20)}$$

with Q_{10} = 2, and T = temperature (°C; measured at the seabed at each site). Nematode total respiration rates (µg C 10 cm^{-2} d^{-1}) were computed as the product of R with total nematode biomass (µg C 10 cm^{-2}). Total nematode biomass (µg C 10 cm^{-2}) was obtained by multiplying for each station the arithmetic mean of B with total density (ind. 10 cm^{-2}).

2.5 Data analysis

Geographical (longitudinal and bathymetric) and environmental trends (relationship with POC, SVI and SED) in nematode diversity, as well as the relationship between diversity and total respiration were evaluated with (multiple) linear regression. To account for region-specific bathymetric patterns in diversity, we included an interaction term between depth and longitude in our models. This interaction term was, however, never significant. Regressions of total respiration against diversity were run both with (accounting for total biomass) and without total nematode biomass (not accounting for total biomass) as an independent variable to evaluate confounding biomass effects on respiration rates. Relationships amongst taxon and functional diversity indices were explored with Spearman rank correlations, corrected for multiple testing using the method of Benjamini and Yekutieli (2001). Here we used correlation analysis because we did not assume a relationship of functional dependence between these variables (Zar 2010).

For the linear regression analysis, partial residual plots were used to examine the linearity of the relationship between the dependent and independent variables (Moya-Laraño and Corcobado 2008). The other assumptions of linear regression (homogeneity of variances, normally distributed residuals, absence of outliers) were checked visually on the basis of the residual plots (Zuur et al. 2010). Additionally, normality of the residuals and homogeneity of variances were tested using a Shapiro-Wilk test and a Non-constant Variance Score Test, respectively. When the variance inflation factors of the independent variables exceeded 5, indicative of multicollinearity, variables were centered (i.e. from each observation the average of the variable was subtracted). When assumptions were not met, independent variables or the dependent variable were $\log_e$-transformed or squared. When a unimodal pattern was evident for an independent variable, the quadratic term of this variable was added. The minimal adequate model was selected on the basis of the P-values of the partial regression tests. Models with and without quadratic terms were compared with an ANOVA "lack of fit" test. Our samples were clustered per region (Fig. 1) and thus we checked for spatial autocorrelation which can lead to an increased chance of type I errors (i.e. falsely rejecting the null hypothesis) (Dormann et al. 2007). We conducted global Moran's I tests on the residuals of all linear regression models (Plant 2012), which showed no significant spatial autocorrelation. Nevertheless, to account for the dependencies between samples collected in the same region, we fitted a linear mixed effects (LME) model with region as a random factor and the aforementioned independent variables as fixed effects to our data. When the likelihood ratio (LR) test indicated that the random region effect was not statistically significant (Pinheiro and Bates 2000), this term was removed and we proceeded with the linear regression model (LM). When the random region effect resulted in a significant improvement of the model (indicating a significant influence of spatial

autocorrelation on LM results), however, we interpreted the results of the LME. Adjusted R^2 (R^2_{adj}) and marginal R^2 (R^2_m: variance explained by the fixed effects; Nakagawa and Schielzeth 2013) denote the goodness of fit of the linear models and the linear mixed effect models, respectively.

All statistical analyses were conducted in R (R Core Team 2012) with the packages car (linear regression assumption checks; Fox and Weisberg 2011), spdep (test for spatial autocorrelation, Bivand 2012), psych (correlation analysis with a correction for multiple testing; Revelle 2012), MuMIn (calculation of R^2_m; Barton 2013) and nlme (fit LME models; Pinheiro et al. 2012). Graphs were made with the ggplot2 package (Wickham 2009). When two independent variables had a significant effect on nematode diversity or respiration in the LM, the isolated effect of each variable was shown using partial regression plots. We added the means of the raw variables to the residuals displayed on the axes to place these on the same scale as the raw variables (Moya-Laraño and Corcobado 2008).

3 Results

3.1 Longitudinal and bathymetric patterns in nematode diversity

The results of the regression analyses examining the longitudinal and bathymetric trends in nematode diversity are shown in Table 2. The phylogenetic diversity index Δ^+, the diversity of life history strategies (c-p diversity), trophic diversity (TD), the maturity index (MI) and the index of biomass diversity (BD) showed no trend with water depth or longitude. Shannon-Wiener diversity H' (Fig. 2A) and expected genus richness EG(20) (Fig. 2B) both declined with water depth, but showed no longitudinal trend. Values of Pielou's evenness J' (Fig. 2C), taxonomic distinctness based on quantitative data Δ^* (Fig. 2D) and taxonomic evenness $1/\Lambda^+$ (Fig. 2E) increased from west to east, but remained constant with water depth. The ellipse plots constructed with the TAXDEST routine showed that Δ^+ and Λ^+ were not mechanistically related, meaning they were measuring different properties of the taxonomic tree (data not shown).

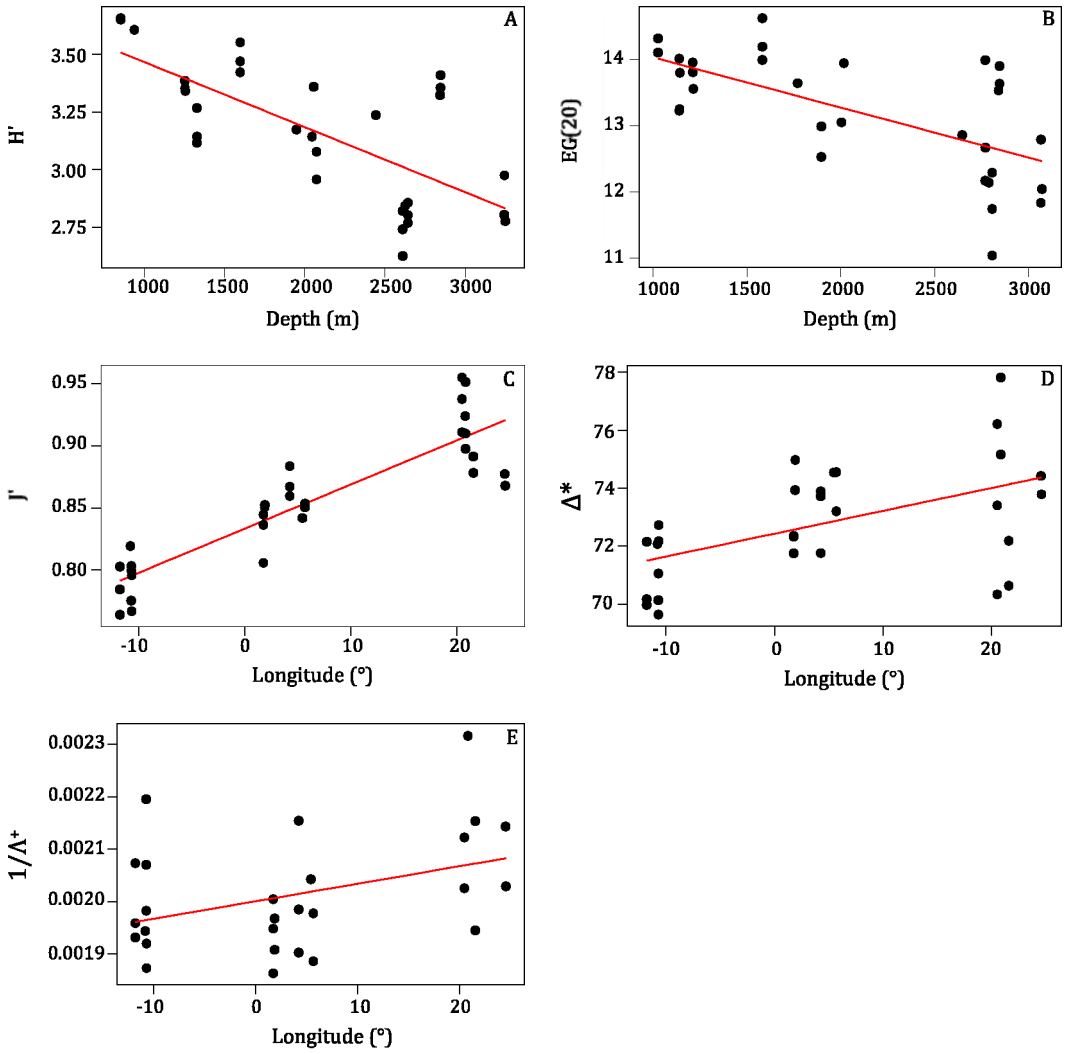

Fig. 2. *Bathymetric and longitudinal trends in nematode diversity. For H' and EG(20), partial regression plots were constructed to show the isolated effect of water depth, while the other plots show marginal regressions. H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, J': Pielou's evenness, Δ*: average taxonomic distinctness based on quantitative data, 1/Λ+: taxonomic evenness. The goodness of fit of these regressions is indicated in Table 2.*

***Table 2**. Results of linear models (LM), the likelihood ratio test (LR test) and the linear mixed effect models (LME) for the regression of water depth and longitude against nematode diversity. For the independent variables depth and longitude (long), the estimated size of the effect and the associated P value are given. For EG(20) both long and long^2 were retained in the model. For H' and BD, long was squared (long^2) to comply with regression assumptions. H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, J': Pielou's evenness, TD: trophic diversity, c-p diversity: diversity of c-p (life history) classes, MI: maturity index, Δ$^+$: average taxonomic distinctness based on presence-absence data, Δ*: average taxonomic distinctness based on quantitative data, 1/Λ$^+$: taxonomic evenness, BD: biomass diversity. G.o.f. = goodness of fit, LR= likelihood ratio.*

	LM			LR test	LME		
	G.o.f.	**Depth**	**Longitude**		**G.o.f.**	**Depth**	**Longitude**
H'	R^2_{adj} = 0.72 P < 0.001	-2.8×10^{-4} P < 0.001	long^2: -9.4×10^{-4} P < 0.001	LR = 37.09 P < 0.001	R^2_m= 0.44	-1.8×10^{-4} P < 0.001	Long^2: -7.3×10^{-4} P = 0.06
J'	R^2_{adj} = 0.79 P < 0.001	- -	3.6×10^{-3} P < 0.001	LR = 9.81 P < 0.01	R^2_m= 0.66	- -	3.1×10^{-3} P = 0.001
EG(20)	R^2_{adj} = 0.53 P < 0.001	-7.4×10^{-4} P < 0.001	long: 3.5×10^{-2} ; long^2: -2.8×10^{-3} long: P = 0.01; long^2: P < 0.01	LR = 4.34 P < 0.05	R^2_m= 0.43	-6.7×10^{-4} P < 0.001	long: 3.6×10^{-2}; long^2: -2.7×10^{-3} long: P = 0.25; long^2: P = 0.13
TD	R^2_{adj} = 0.17 P = 0.01	- -	8.0×10^{-3} P = 0.01	LR = 5.60 P < 0.05	R^2_m= 0.20	- -	8.8×10^{-3} P = 0.15
C-p div	R^2_{adj} = 0.09 P = 0.06	-2.6×10^{-5} P = 0.06	- -	LR = 9.62 P < 0.01	R^2_m= 0.008	-7.0×10^{-6} P = 0.54	- -
MI	R^2_{adj} = 0.09 P = 0.06	- -	3.2×10^{-3} P = 0.06	- -	-	- -	- -
Δ*	R^2_{adj} = 0.25 P < 0.01	- -	0.1 P < 0.01	LR = 0.81 P = 0.37	-	- -	- -
Δ$^+$	R^2_{adj} = 0.08, P = 0.07	6.6×10^{-4} P = 0.07	- -	- -	-	- -	- -
1/Λ$^+$	R^2_{adj} =0.12 P < 0.05	- -	3.3×10^{-6} P < 0.05	LR = 0.69 P = 0.41	-	- -	- -
BD	R^2_{adj} = 0.07 P = 0.08	- -	long^2: -2.7×10^{-4} P = 0.08	- -	-	- -	- -

3.2 Environmental drivers of nematode diversity

Indices J', c-p diversity, $1/\Lambda^+$ and BD did not relate to seasonal variability in POC flux (SVI), sediment particle size diversity (SED) or seafloor POC flux (POC). Both H' (Fig. 3A) and EG(20) (Fig. 3B) showed a positive linear relationship with $\log_e$-transformed POC. TD related inversely with SED (Fig. 3C), whereas taxonomic distinctness based on presence-absence (Δ^+, Fig. 3G) and quantitative data (Δ^*, Fig. 3E) increased with increasing SED. MI (Fig. 3D) and Δ^* (Fig. 3H) were influenced negatively by SVI, and Δ^+ declined with increasing POC values (Fig. 3F).

3.3 Relationship between nematode taxon and functional diversity

After correcting for multiple testing, expected genus richness EG(20) correlated positively with c-p diversity (Spearman rank, $r = 0.63$, $P < 0.01$; Fig. 4). The other taxon diversity indices did not relate to any of the functional diversity measures.

***Table 3**. Results of linear models (LM), the likelihood ratio(LR) test and linear mixed effect models (LME) for the regression of sediment particle size diversity (SED), seafloor particulate organic carbon flux (POC) and seasonal variability in POC flux (SVI) against nematode diversity. The estimated size of the effect and the associated P value are given per independent variable. For the regressions against H', J', EG(20) and c-p diversity (c-p div), POC was log_e-transformed to comply with the assumptions of linear regression. For c-p diversity, also SVI was log_e-transformed to comply with assumptions. H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, J': Pielou's evenness, TD: trophic diversity, c-p diversity: diversity of c-p (life history) classes, MI: maturity index, Δ+: average taxonomic distinctness based on presence-absence data, Δ*: average taxonomic distinctness based on quantitative data, 1/Λ+: taxonomic evenness, BD: biomass diversity. G.o.f. = goodness of fit*

	LM				LR test	LME			
	G.o.f.	**SED**	**POC**	**SVI**		**G.o.f.**	**SED**	**POC**	**SVI**
H'	R^2_{adj} = 0.70 P < 0.001	-0.72 P < 0.01	log(POC): 0.68 P < 0.001	- -	LR = 38.81 P < 0.001	R^2_m= 0.42	-0.27 P = 0.07	log(POC): 0.45 P < 0.001	- -
J'	R^2_{adj} = 0.70 P < 0.001	- -	log(POC): -0.06 P < 0.001	-0.21 P = 0.001	LR = 17.19 P < 0.001	R^2_m=	- -	log(POC): 0.003 P = 0.80	-0.27 P = 0.09
EG(20)	R^2_{adj} = 0.47 P < 0.001	- -	log(POC): 1.46 P < 0.001	-5.29 P < 0.001	LR = 5.65 P < 0.05	R^2_m= 0.44	- -	log(POC): 1.50 P < 0.001	-4.87 P = 0.06
TD	R^2_{adj} = 0.26 P < 0.01	-0.68 P < 0.01	- -	- -	LR = 2.95 P = 0.09	-	- -	- -	- -
C-p div	R^2_{adj} = 0.22 P = 0.01	-0.68 P < 0.01	log(POC): 0.06 P < 0.01	log(SVI): -0.07 P = 0.01	LR = 7.15 P < 0.01	R^2_m= 0.08	- -	log(POC): 0.02 P = 0.39	log(SVI): -0.05 P = 0.42
MI	R^2_{adj} = 0.19 P < 0.01	- -	- -	-0.53 P < 0.01	LR = 0.17 P = 0.68	-	- -	- -	- -
Δ*	R^2_{adj} = 0.38 P < 0.001	4.20 P < 0.05	- -	-13.90 P < 0.001	LR = 020 P = 0.65	-	- -	- -	- -
Δ+	R^2_{adj} = 0.23 P = 0.01	4.51 P < 0.01	-0.25 P < 0.05	- -	LR = 1.12×10^{-8} P = 0.99	-	- -	- -	
1/Λ+	R^2_{adj} = 0.03 P = 0.17	- -	-1.24×10^{-5} P = 0.17	- -	LR = 0.69 P = 0.41	-	- -	- -	
BD	R^2_{adj} = 0.03 P = 0.17	0.23 P = 0.17	- -	- -	- -	-	- -	- -	

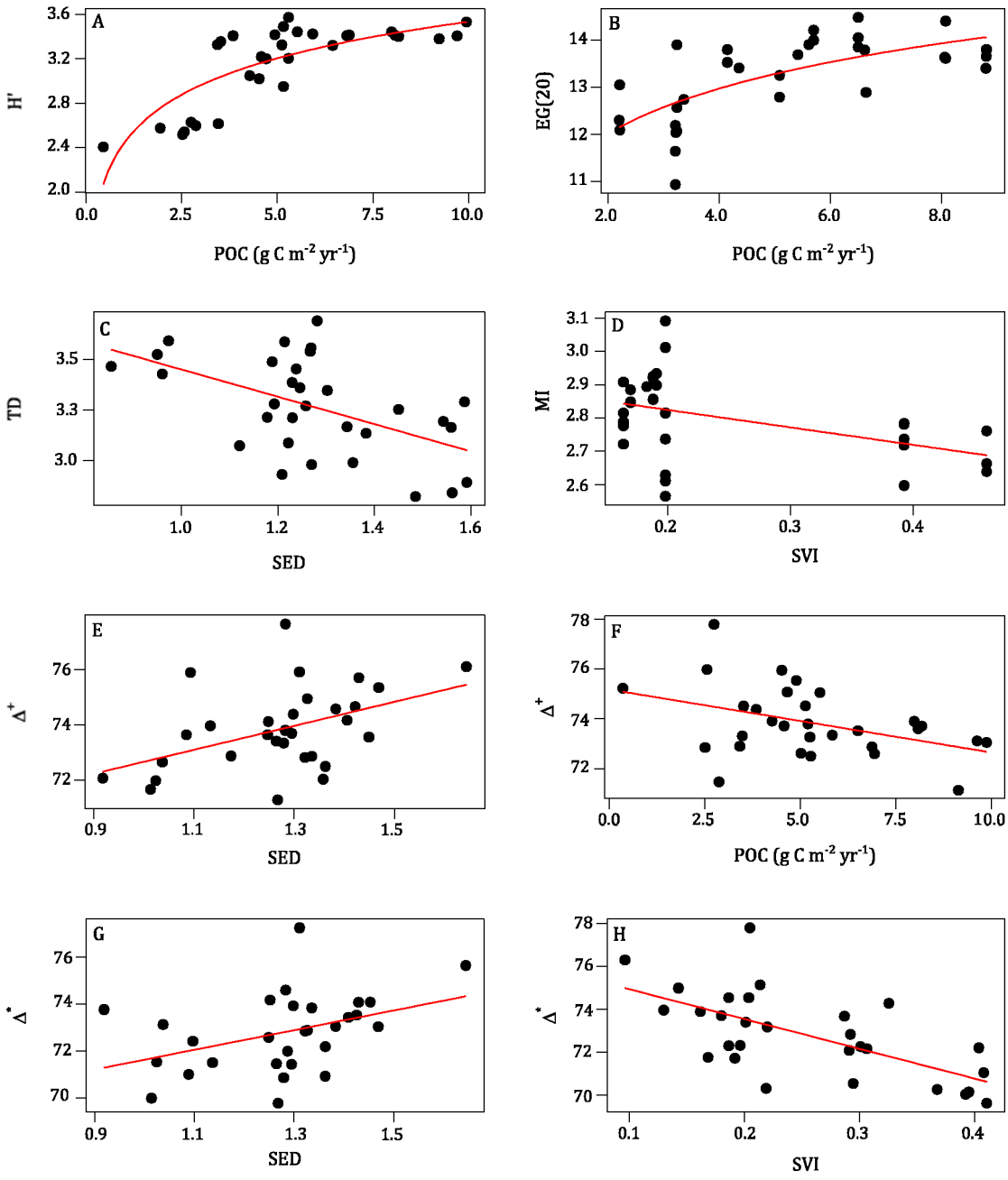

Fig. 3. *Environmental drivers of nematode diversity. Plots A, B, E-H show partial regression results, whilst plots C and D show marginal regressions. POC: seafloor particulate organic carbon flux, SED: sediment particle size diversity, SVI: seasonal variability in POC flux, H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, TD: trophic diversity, MI: maturity index, Δ⁺: average taxonomic distinctness based on presence-absence data, Δ*: average taxonomic distinctness based on quantitative data. The goodness of fit of these regressions is shown in Table 3.*

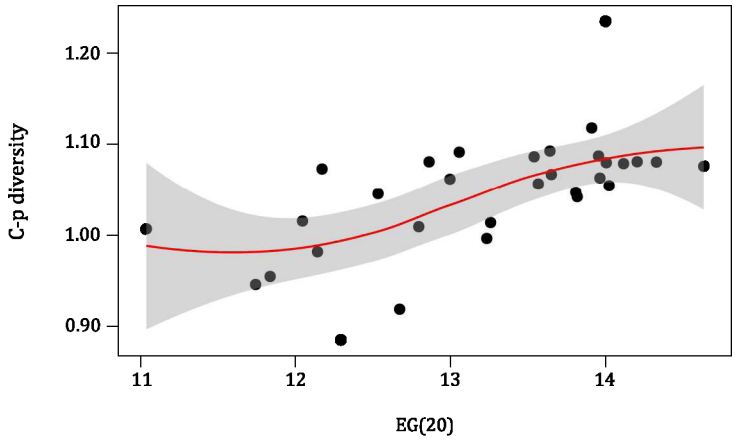

Fig. 4. *Significant Spearman rank correlation between taxon diversity (EG(20)) and functional diversity (c-p diversity) of nematodes. The red line and associated grey zone represent a LOESS smoother and the 95 % confidence interval, respectively. EG(20): expected genus richness for a sample of 20 individuals, c-p diversity: diversity of c-p (life history) classes.*

3.4 Effect of diversity on respiration rates in nematode communities

In the regressions of diversity against total respiration, only indices H' and BD had a statistically significant effect (Table 4). H' showed a positive linear relation with $\log_e$-transformed total respiration, and squared BD related positively and linearly with total respiration (Fig. 5A-B). After accounting for biomass (by including this variable in the regression against respiration), only taxon diversity indices H' and EG(20) had a positive effect on nematode total respiration (Table 5, Fig. 5C-D).

Table 4. *Results of linear models (LM), the likelihood ratio (LR) test and linear mixed effect models (LME) for the regression of nematode diversity against total respiration. The estimated average size of the effect and the associated P value are given per diversity index. For H' and EG(20), respiration (resp) was log_e-transformed to comply with the assumptions of linear regression. J' and BD were log_e-transformed and squared, respectively, to comply with assumptions. H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, J': Pielou's evenness, TD: trophic diversity, c-p diversity: diversity of c-p (life history) classes, MI: maturity index, Δ^+: average taxonomic distinctness based on presence-absence data, Δ^*: average taxonomic distinctness based on quantitative data, $1/\Lambda^+$: taxonomic evenness, BD: biomass diversity. G.o.f. = goodness of fit.*

	LM		LR test	LME	
	G.o.f.	**Effect of diversity**		**G.o.f.**	**Effect of diversity**
H'	R^2_{adj} = 0.71 P < 0.001	log(resp): 2.45 P < 0.001	LR = 4.25 P < 0.05	R^2_m = 0.51	1.80 P < 0.001
J'	R^2_{adj} = 0.35 P < 0.001	log(J'): -1.35 P < 0.001	LR = 6.99 P < 0.01	R^2_m = 0.02	-0.99 P = 0.09
EG(20)	R^2_{adj} = 0.24 P < 0.01	log(resp): -9.85 P <0.01	LR = 26.43 P < 0.001	R^2_m = 0.02	Log(resp): 0.17 P = 0.23
TD	R^2_{adj} = 0.00 P = 0.37	-0.10 P = 0.37	- -	-	- -
C-p div	R^2_{adj} = 0.10 P = 0.05	0.88 P = 0.05	LR = 11.10 P < 0.001	R^2_m = 0.0008	0.08 P = 0.87
MI	R^2_{adj} = 0.00 P = 0.70	-0.08 P = 0.70	- -	-	- -
Δ*	R^2_{adj} = 0.00 P = 0.56	0.005 P = 0.56	- -	-	- -
Δ⁺	R^2_{adj} = 0.02 P = 0.23	0.01 P = 0.23	- -	-	- -
1/Λ⁺	R^2_{adj} = 0.00 P = 0.37	-52.08 P = 0.37	- -	-	- -
BD	R^2_{adj} = 0.40 P < 0.001	BD^2: 0.14 P < 0.001	LR = 8.21 P < 0.01	R^2_m = 0.18	0.09 P < 0.01

***Table 5**. Results of linear models (LM), and the likelihood ratio (LR) test for the regression of nematode diversity and total nematode biomass against total respiration. Since the LR test was never significant, only the LM results were interpreted. The estimated average size of the effect and the associated P value are given for biomass and each diversity index. H': Shannon-Wiener diversity, EG(20): expected genus richness for a sample of 20 individuals, J': Pielou's evenness, TD: trophic diversity, c-p diversity: diversity of c-p (life history) classes, MI: maturity index, Δ⁺: average taxonomic distinctness based on presence-absence data, Δ*: average taxonomic distinctness based on quantitative data, 1/Λ⁺: taxonomic evenness, BD: biomass diversity. G.o.f. = goodness of fit.*

	LM			LR test
	G.o.f.	**Effect of biomass**	**Effect of diversity**	
H'	R^2_{adj} = 0.66 P < 0.001	0.02 P < 0.001	0.15 P < 0.01	LR = 3.56 P = 0.06
J'	R^2_{adj} = 0.69 P < 0.001	log(bio): 0.12 P < 0.001	0.57 P = 0.23	- -
EG(20)	R^2_{adj} = 0.63 P < 0.001	0.02 P < 0.001	0.05 P = 0.01	LR = 2.85 P = 0.09
TD	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.10 P < 0.001	-0.03 P = 0.65	- -
C-p div	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.10 P < 0.001	-0.007 P = 0.98	- -
MI	R^2_{adj} = 0.67 P < 0.001	log(bio): 0.10 P < 0.001	-18.3×10^{-4} P = 0.99	- -
Δ*	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.10 P < 0.001	-0.003 P = 0.50	- -
Δ⁺	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.10 P < 0.001	-0.005 P = 0.45	- -
1/Λ⁺	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.10 P < 0.001	13.12 P = 0.70	- -
BD	R^2_{adj} = 0.68 P < 0.001	log(bio): 0.09 P < 0.001	0.09 P = 0.36	- -

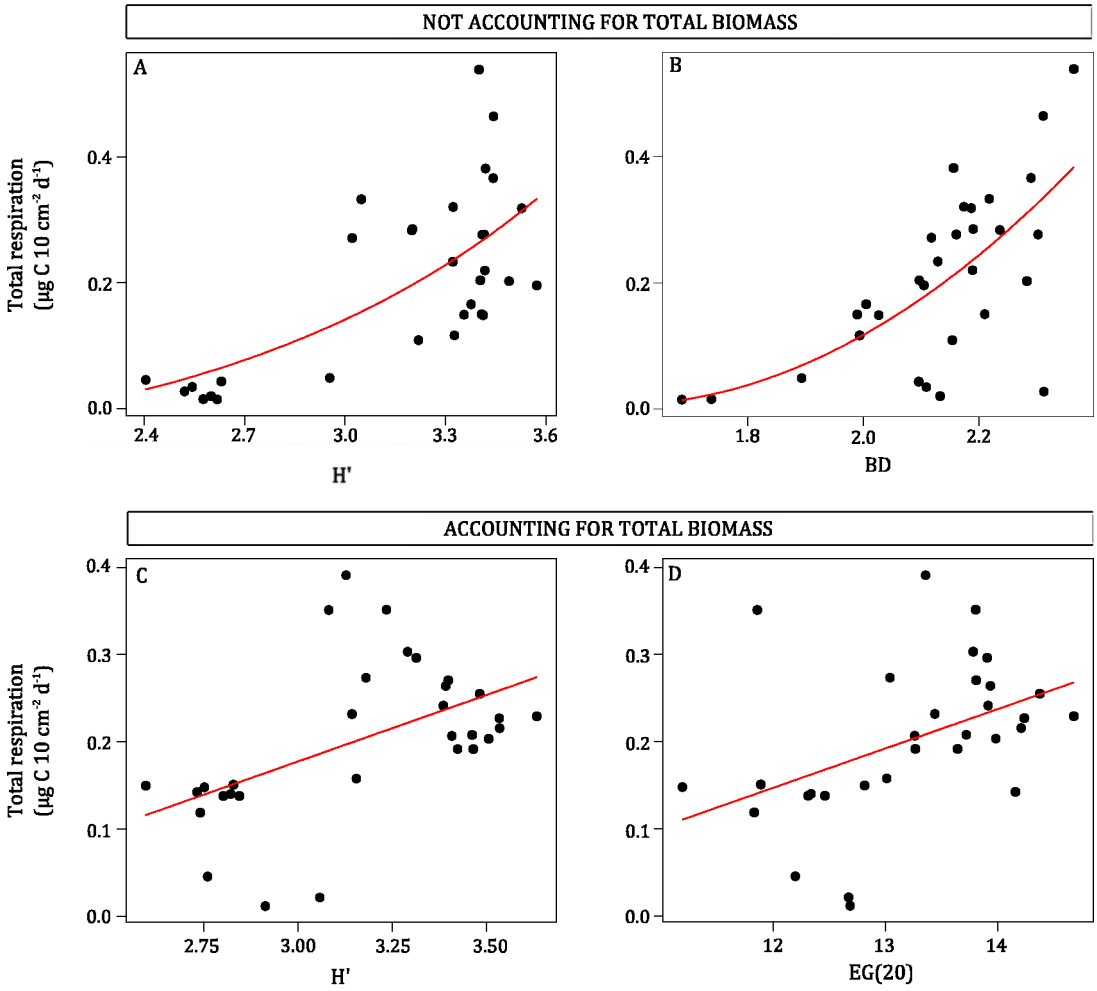

Fig. 5. *Relationship between diversity and total respiration in nematode communities. Plots A and B show the marginal regressions of diversity against respiration (not accounting for total nematode biomass), while plots C and D show the partial regressions of diversity against respiration (accounting for total nematode biomass). H': Shannon-Wiener diversity, BD: biomass diversity, EG(20): expected genus richness for a sample of 20 individuals.*

4 Discussion

4.1 Longitudinal and bathymetric patterns in nematode diversity

One of the first steps in unravelling the drivers of biodiversity constitutes the description of broad-scale geographical patterns. Nematode genus diversity, measured as Shannon-Wiener diversity and expected genus richness EG(20), did not change along the longitudinal axis between the Galicia Bank (GB) region, in the northeast Atlantic, and the eastern Mediterranean. In contrast, similar studies based on nematode species found a significant decrease in diversity between the northeast Atlantic and the southern Adriatic Sea (Danovaro et al. 2009a) and

alongside the longitudinal axis in the Mediterranean basin (Danovaro et al. 2008b, 2009b, 2010). Even though genus richness remained relatively constant along the longitudinal axis, generic (J') and taxonomic evenness ($1/\Lambda^+$), as well as the average taxonomic distinctness amongst individuals (Δ^*, quantitative data) increased towards the east. Both a higher generic evenness and a greater distance between the more abundant genera in the taxonomic tree can result in higher values of Δ^*. Hence, compared to nematode communities in the east, nematode assemblages in the west were characterized by a more imbalanced taxonomic tree (more unequal spread of genera across the taxonomic tree) and a more uneven spread of individuals over the different genera, whether or not in combination with a lower taxonomic distinctness (or higher relatedness) between the dominant genera.

We observed a decline in taxon diversity indices H' and EG(20) with increasing water depth, contrasting with numerous previous reports of a unimodal diversity-depth trend for multiple benthic taxa (Stuart et al. 2003, Menot et al. 2010b, Rex and Etter 2010). However, the depth range covered here is relatively narrow (1026-3072 m, Table 1) and diversity may be depressed at shallower depths. In other words, it is possible that our samples fell within the descending section of the unimodal bathymetric diversity curve. Danovaro et al. (2010), who considered a larger depth range than us, discovered a hump-shaped bathymetric trend in nematode species diversity, albeit only in the eastern Mediterranean basin. In contrast, Tselepides et al. (2000) described a decrease in macrofaunal diversity between 40 and 1570 m water depth along the Cretan continental margin. Rex and Etter (2010) speculated that when nutrient loadings become very scarce, as is the case in the Mediterranean, there is a shift from a fully unimodal diversity-depth curve towards just the ascending portion (i.e. positive association between productivity and diversity). Alternatively, the absence of a peak in diversity at intermediate water depths may be related to the unusually warm (13 °C) and isothermal water column in the Mediterranean (Tyler 2003). Unimodal diversity-depth trends are generally found in open oceans like the Atlantic and the Pacific where temperature decreases rapidly (to barely a few degrees) with depth. Opposed to Danovaro et al. (2009a) and Danovaro et al. (2010), bathymetric diversity patterns did not vary between the different regions that were sampled.

The divergence between the present results and those of Danovaro et al. (2008b) and Danovaro et al. (2010) regarding bathymetric and longitudinal trends in nematode diversity may be attributed to the different taxonomic levels (genera and species, respectively) and sediment depth strata that were investigated (0-10 and 0-1 cm, respectively). For deep-sea nematodes inhabiting the Kenyan continental margin, spatial patterns in genus diversity differed substantially from species diversity trends (Muthumbi et al. 2011). In contrast, Leduc et al. (2012a) found very comparable environmental trends in species and genus diversity at the

continental slope of New Zealand. The difference in species and genus patterns along the Kenyan margin (Muthumbi et al. 2011) were attributed to the unequal distribution of the number of species per genus, with some genera consisting of many species (e.g. *Acantholaimus*, De Mesel et al. 2006) and many genera consisting of a few species.

4.2 Environmental drivers of nematode diversity

Productivity and its mediation of biological interactions has been proposed as a potential mechanism for the commonly observed unimodal bathymetric and linear latitudinal diversity gradients in deep-sea sediments (Levin et al. 2001, Stuart et al. 2003). Here, the magnitude of seafloor POC flux had a positive impact on nematode taxon diversity, measured as H' and EG(20), consistent with earlier work on polychaetes (Glover et al. 2002) and nematodes (Lambshead et al. 2002) from the abyssal central Pacific. It was shown that seafloor POC flux declines from the northeast Atlantic to the eastern Mediterranean (not considering the seamount station) and with water depth (Pape et al. in press; Chapter 4), and hence this environmental factor may partly explain the observed bathymetric decline in taxon diversity (see 4.1). The detection of a positive association between diversity and productivity does not necessarily negate the existence of a hump-shaped productivity-diversity curve. The productivity gradient considered in this study may occupy only the left, ascending limb of the unimodal diversity-productivity curve (Levin et al. 2001). In support, in the Atlantic and the Gulf of Mexico, Menot et al. (2010b) found a diversity peak at an organic carbon flux of 10-15 g C m^{-2} yr^{-1} for several macrofaunal phyla, which is the maximum value of seafloor POC flux observed in our study area. Opposed to the traditionally employed diversity measures (i.e. Shannon-Wiener diversity and expected genus richness), average taxonomic distinctness (based on presence-absence data, Δ^+) was inversely related to seafloor POC flux. Hence, along our transect, areas characterized by higher POC input harboured a higher number of relatively closely related genera, whereas areas receiving less POC were inhabited by less, but more distantly related genera. It seems that a high POC flux regime is favouring a higher number of nematode genera that are relatively closely related and consequently exhibit similar properties that allow them to outcompete other genera or withstand predation pressure by larger fauna.

In the present study, higher seasonality in surface productivity (SVI) was reflected in a reduced nematode maturity index, governed by the increased contribution of colonisers or opportunists to nematode standing stock (Bongers et al. 1991, Bongers and Ferris 1999). It is believed that these nematodes can cope better with variable environmental conditions such as those induced by pulsed organic matter input. Nematode communities in more seasonal regions displayed also

lower average taxonomic distinctness (based on quantitative data, Δ^*). This finding suggests that the ability to maintain high abundances under a more pulsed organic matter loading may be confined to certain taxonomic groups. Clearly, our results imply that both the magnitude and the seasonality of seafloor POC flux impact the average taxonomic distinctness within nematode communities, which may be translated to a greater functional distinctness. However, since taxonomic distinctness may be governed by a variety of factors, such as biogeography, environmental factors, habitat characteristics, and stress (Warwick and Clarke 1995, Warwick and Clarke 1998, Mouillot et al. 2005, Leira et al. 2009, Xu et al. 2011, Bevilacqua et al. 2012), more research into life history strategies, niche requirements and taxon interactions are needed to fully understand the patterns observed here.

Unlike Leduc et al. (2011) (nematode species and genera) and Etter and Grassle (1992) (macrofaunal species), we did not detect an effect of sediment heterogeneity (SED) on nematode genus diversity. We did, however, observe that more heterogeneous sediments harboured nematode assemblages with a higher taxonomic breadth, and possibly a higher functional divergence. The higher habitat heterogeneity may favour the co-existence of more taxonomically dissimilar taxa, with their distinct specific niche requirements. Possibly, the high genus diversity observed by Leduc et al. (2011) coincided with high taxonomic distinctness. Leduc et al. (2011) found no effect of SED on nematode trophic diversity (TD), whereas we uncovered an inverse relationship between SED and TD. It should be stressed that the trends described here do not imply causal relationships, and that the decrease in nematode diversity with increasing SED may be driven by a confounding, unmeasured environmental factor. The differential calculation of sediment heterogeneity hampers the comparison between our study and that of Leduc et al. (2011). Whereas we considered ten different grain size classes (see 2.2), Leduc et al. (2011) used only five sediment grain size classes in their calculation of SED without subdividing the mud fraction (< 63 μm) of the sediment. Finally, note that our SED calculation and that of Etter and Grassle (1992) and Leduc et al. (2011) was based on dry-sieved sediment fractions and it is possible that this measure of particle diversity is not representative for the *in situ* size distribution of aggregated sediment particles (Snelgrove and Butman 1994, Levin et al. 2001).

4.3 Link between nematode taxon and functional diversity

Our results showed that nematode communities with higher taxon diversity were characterized by a greater variety of life history strategies (higher c-p diversity). If higher c-p diversity governs enhanced resistance against environmental fluctuations or resilience following disturbance, this may point to a positive long-term effect of taxon diversity on ecosystem functioning (Loreau

2000). We found no links between the other taxon and functional diversity measures, and hence the presence of a relationship between taxon and functional diversity depended on the type of functional traits considered. However, the functional diversity indices computed here might not encompass the entire array of functions performed by the nematode community. For instance, the feeding type classification scheme based on buccal morphology (Wieser 1953) may be too coarse to represent a truthful proxy for resource partitioning. In support, De Mesel et al. (2003) observed that coastal nematode species belonging to the same feeding guild had a differential influence on cordgrass decomposition rates. The validity of our results concerning the association between taxon and functional diversity in other oceanographic regions remains to be tested as it is partly determined by the degree of redundancy and singularity within a community, as well as by biogeography and biotic interactions (Hooper et al. 2002, Naeem and Wright 2003).

4.4 Effect of diversity on respiration rates in nematode communities

The present study showed that deep-sea nematode communities with higher Shannon-Wiener genus diversity (H') or higher individual biomass diversity (BD) showed higher total respiration rates, and that the influence of both diversity indices was more pronounced at higher values (as inferred from the exponential and power function describing the dependency of respiration on H' and BD, respectively). However, when differences in total nematode biomass between samples were accounted for, we only observed a positive linear relationship between taxon diversity (measured as H' and expected genus richness EG(20)) and total respiration. In other words, nematode communities with the same standing stock showed different respiration rates when genus diversity, but not biomass diversity, differed. Hence, the positive impact of BD on respiration could be attributed to the positive covariance between total biomass and the diversity in individual biomass.

More diverse nematode assemblages may mineralise more carbon when the co-existence of more genera results in a more complete utilization of all different carbon sources. Consequently, the linear form of the relation between expected genus richness and total respiration may indicate that all genera contributed more or less equally to the decomposition and mineralisation of organic matter, which is in line with the rivet hypothesis (Lawton 1994, Naeem et al. 1995). Contrary to expectations, we found no proof for functional diversity as a mechanistic link between taxon diversity and ecosystem functioning since none of the functional diversity indices (including taxonomic or phylogenetic diversity) related to both genus diversity and total respiration. Several points can be raised to explain the lack of a significant association

between the functional diversity indices and ecosystem functioning. First of all, as mentioned in section 4.3, the metrics computed here may not adequately represent true functional diversity. The diversity in diet composition amongst nematode genera may not be captured by the trophic diversity index. Secondly, the functional diversity measures used here are perhaps not important for the ecosystem function under study, but they may well be for other functions performed by nematodes. For instance, a nematode community which comprises a wide variety of differently sized individuals (high BD) may create more diversified micro-burrow networks within the sediment. This type of cryptobioturbation and bioirrigation may in turn stimulate small-scale, yet important biogeochemical processes (Aller and Aller 1992, Pike et al. 2001), resulting in elevated carbon mineralisation by the entire benthic community. A more relevant measure of functional diversity to nematode carbon mineralisation may be the diversity in digestive systems, mirrored in taxon diversity. Thirdly, it is possible that environmental conditions influencing both functional diversity and respiration rates vary among sites, resulting in an absence of an across-site pattern even when significant biodiversity effects exist within each locale (Cardinale et al. 2000, Loreau 2000, Hiddink et al. 2009). A fourth point is that total nematode respiration was here estimated on the basis of total nematode biomass and temperature (de Bovée and Labat 1993) and may not truthfully reflect *in situ* respiration by the nematode community. Environmental factors other than temperature (Braeckman et al. in press) and biotic interactions (De Mesel et al. 2006) may influence nematode carbon processing rates.

Phylogenetic diversity may serve as a proxy for functional diversity when related taxa are functionally similar (Cadotte et al. 2008, 2009). The present study did not demonstrate a significant effect of phylogenetic diversity on total respiration for deep-sea nematodes. The lack of a relation between phylogenetic diversity and ecosystem functioning may be because (1) functionally important traits do not have a strong phylogenetic signal (or in other words, closely related taxa do not have similar functional trait values), (2) the signal is reduced because of community assembly, or (3) traits that determine dominance are not important for the function under study (Srivastava et al. 2012). The fact that we found no link between taxon and phylogenetic diversity, whereas total respiration was affected positively by taxon diversity, implies that the phylogenetic diversity indices used here do not serve as good surrogates for the diversity of traits important for total respiration (such as feeding behaviour and/or the digestive apparatus).

The different form of the biodiversity-function curve in the present study (linear) and that of Danovaro et al. (2008a) (exponential) is in all probability related to the different measures that were used to define ecosystem functioning (nematode respiration rates vs. prokaryote biomass

and production, bacterial organic matter decomposition and total faunal biomass, respectively). As demonstrated for shallow marine and terrestrial systems, different ecosystem processes or properties can respond very dissimilarly to changes in biodiversity (Naeem et al. 1995, Bolam et al. 2002). Moreover, note that Danovaro et al. (2008a) considered species and not genera (except for the North Atlantic), and that ecological facilitation, which was proposed as the mechanism underlying the exponential relation, may only be discernible at the species level.

We assumed that respiration is dependent on diversity in nematode communities. However, significant regressions do not necessarily imply causation. If both diversity and respiration are influenced by the same environmental factor(s), this would also result in a significant relationship. Temperature promotes respiration rates and, at least for ophiuroids (O'Hara and Tittensor 2010) and mollusks (Tittensor et al. 2011), also biodiversity. Along our transect, higher POC deposition (food availability) resulted in higher standing stock and thus higher respiration rates (Pape et al. in press, Chapter 4), but also allowed for more taxa to attain viable population sizes. However, the fact that communities with equal biomass (suggesting equal food availability) with differing diversity showed differing respiration rates indicates that food availability is not the sole factor governing the significant diversity-function relation. Experimental studies, employing *in situ* respiration as an ecosystem function, are needed to verify and elucidate the mechanism(s) behind the observed diversity-function relation.

Since climate change is already affecting the pattern of POC flux to the deep-sea bottom and will continue to do so (Smith et al. 2008), the links between the magnitude and variability in POC flux and taxon diversity, on the one hand, and between taxon diversity and ecosystem functioning, on the other hand, suggests that this global phenomenon will modify, or already is modifying, the functioning of deep-sea ecosystems.

4.5 Conclusions

Several of the nematode diversity indices that we calculated displayed significant bathymetric and longitudinal patterns, which may be partly ascribed to variations in the rate and seasonality of organic matter deposition, and in sediment heterogeneity. Accounting for confounding biomass effects, we observed a positive linear relationship between nematode taxon diversity and nematode carbon mineralisation, estimated from total nematode biomass. The fact that none of the indices of functional diversity, including phylogenetic diversity, related to both taxon diversity and mineralisation rates suggests that these indices did not encompass the entire array of nematode functional traits that are of importance to nematode carbon mineralisation. Our results suggest potential repercussions of climate change on nematode carbon mineralisation

rates in the deep sea. In light of the progressive change in global climatic patterns, it is clear that we urgently need to improve our knowledge regarding the functions that nematodes perform within deep-sea sedimentary ecosystems and how these are affected by evolutionary processes.

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Chapter 6

General discussion and recommendations for future research

This last chapter integrates the most important scientific results of the present doctoral research. Here, I mainly discuss the environmental determinants of patterns in the composition, diversity and function of deep-sea nematode communities along a longitudinal transect in southern European deep-sea basins, and on a low-activity mud volcano in the Gulf of Cádiz. At the end, I highlight the main conclusions drawn from this work and summarize the most important caveats in our knowledge that need to be addressed. This is accompanied by several suggestions for future research.

1 Environmental drivers of deep-sea nematode community attributes

In what follows, I will discuss the main findings from the preceding chapters on the environmental drivers of the various structural and functional aspects of nematode assemblages in the deep sea. Some factors were particularly important in either the cold seep or photosynthetically driven habitats investigated, and therefore these two systems were treated separately. Although food items for deep-sea nematodes may constitute other organisms, food availability is here considered as an environmental agent.

1.1 Standing stock

Understanding which factors drive the standing stock (i.e. density and biomass) of (deep-sea) organisms may help to predict their response to natural and anthropogenic changes in their habitat. In both the non-seep sedimentary environments of the Galicia Bank region and the Mediterranean basin (**Chapter 4**), and the seep ecosystem at the Darwin mud volcano in the Gulf of Cádiz (**Chapter 2**), food availability was identified as an important determinant of the

standing stock of deep-sea nematodes. Besides the amount of food, nematode density and biomass were also related to other environmental conditions specific to these two systems.

In the photosynthetically driven benthic habitats of the Galicia Bank (GB) region and the Mediterranean Sea, the amount of food available to deep-sea nematodes was approximated on the basis of modelled seafloor particulate organic carbon (POC) flux data and benthic potential food indicators such as phytopigment, nitrogen and organic matter concentrations. Our results showed a decline in seafloor POC flux and sedimentary phytopigment levels between the GB region and the eastern Mediterranean, which was translated in a west-east reduction in nematode densities and individual biomass. An exception to this trend was the station atop of the GB seamount, which was, despite the elevated primary productivity of the overlying waters, characterized by abnormally low nematode standing stock. This was ascribed to the strong hydrodynamic regime (Duineveld et al. 2004) either through the removal of nutritious organic matter or the physical stress inflicted on the infauna, or both. The reduced bacterial biomass (**Chapter 3**) and the low organic matter, phytopigment and nitrogen concentrations (**Chapters 3 and 4**) at the seamount station support the former hypothesis. Opposed to the GB seamount, the HEBBLE site in the western Atlantic, which frequently experiences benthic storms, is characterized by nematode abundances which are not reduced but fall within the same range as those at nearby tranquil sites (Thistle et al. 1991). The food available at this site is ample, indicated by the high concentrations of organic carbon and nitrogen, the accumulation of fresh diatoms in the numerous relict macrofaunal burrows, as well as by the enhanced microbial biomass relative to other deep-sea localities (Yingst and Aller 1982, Aller and Aller 1986). Sedimentary nitrogen and carbon concentrations at the HEBBLE site were both at least three times higher than those measured at the GB (Aller and Aller 1986). Thistle et al. (1991) argued that at the HEBBLE site, the enhanced nematode growth rates owing to the copious food supply were counteracted by the increased grazing pressure of the unusually abundant macrofauna, resulting in no net change in nematode standing stock. Contrary to the HEBBLE site, macrofaunal densities at the GB seamount were not stimulated by the hydrodynamic regime (Flach et al. 2002) and grazing pressure appears not to be responsible for the low nematode abundances at the seamount station. The comparison between the GB and the HEBBLE site shows that strong bottom currents do not necessarily always depress nematode standing stock, and that other factors, such as food availability, co-determine patterns in nematode abundance and biomass.

As mentioned before, not only nematode densities, but also individual biomass decreased significantly along the gradient of primary productivity between the GB seamount and the more oligotrophic Levantine basin. This trend may be attributed to the west-to-east decline in food

availability, as observed for other deep-sea nematode assemblages (Vanreusel et al. 1995a, Soetaert et al. 2002, Udalov et al. 2005). Additionally, given that the sedimentary mud fraction related negatively with individual body weight, the reduction in individual biomass may be the consequence of the lower number of larger, burrowing specimens which are unable to maintain high densities in the more fluid, finer sediments in the eastern Mediterranean (Udalov et al. 2005).

At the Darwin mud volcano (MV) in the Gulf of Cádiz, seep influenced sediments (0 to 5 m from the seep site) harboured higher nematode densities and biomass than sediments that did not show signs of seepage activity (from 10 m from the seep site onwards), consistent with several previous studies (Olu et al. 1997, Buck and Barry 1998, Robinson et al. 2004, Van Gaever et al. 2006, 2009c). Elevated nematode standing stock at cold seeps may be attributed to the chemosynthesis-related proliferation of microbial biomass and small microvores, which serve as a potential food source. The enhanced nematode community biomass within the seep influenced sediment samples was not only governed by high abundances but also by elevated body weight, which is a further indication of copious amounts of food (Vanreusel et al. 1995a, Soetaert et al. 2002, Udalov et al. 2005). Stable isotope analysis confirmed that thiotrophic carbon was part of the nematode diet up to 10 m from the seep sediments. At the Darwin MV seep site and 2 m away, pore-water profiles of sulphide, sulphate, methane and alkalinity indicated the occurrence of anaerobic oxidation of methane or AOM. This chemical reaction is carried out by a consortium of archaea and sulphate reducing bacteria (Boetius et al. 2000, Knittel and Boetius 2009). The sulphide that is set free by this process can in turn be utilized by sulphur-oxidizing bacteria, like *Beggiatoa* which often form large mats at cold seeps (Buck and Barry 1998, Sahling et al. 2002, Van Gaever et al. 2006, Sommer et al. 2007). At the Darwin MV, however, large bacterial mats were not seen. Maximal nematode densities and biomass were not attained at the seep site, but at a 2 m distance. This may be attributed to the high sulphide levels measured in the seep sediment pore-water (≥ 22 mM), which most likely coincide with very low to zero oxygen levels (not measured), compared to the sulphide-free surface sediments at a 2 m distance. Sulphide is extremely toxic to metazoans since it blocks the enzyme cytochrome c oxidase and thus the oxygen uptake that is required for regular ATP production (National Research Council 1979). Oxygen is an important prerequisite for metazoan life, including nematodes, since it is the final electron acceptor in aerobic metabolism and it is required in the anabolism and catabolism of fatty acids (McMullin et al. 2000, Wetzel et al. 2001). Nonetheless, compared to larger fauna, densities of nematodes are less severely impaired by hypoxia or anoxia owing to the preponderance of a few species that are relatively tolerant to these conditions, though only when these are not permanent (Wetzel et al. 2002, Steyaert et al. 2007, Gambi et al. 2009). Hence, elevated nematode standing stock at and near the Darwin MV seep site reflects the trade-

off between the disadvantage of environmental toxicity and/or anoxia and the advantage of high food availability. At the seep site, food was copious but the toxic environment did not allow for the proliferation of a dense nematode community; at 2 m from the seep site, food was still plentiful but sulphide levels within the top 10 cm of the sediment column dropped to zero, rendering this a benign environment, suitable for less stress-tolerant nematode species. This mechanism may further account for the high spatial variability in nematode densities that has also been observed within other seep localities (i.e. between sub-habitats) (Van Gaever et al. 2006, 2009a, c). However, seep intensity, which was negligible at the Darwin MV (Vanneste et al. 2012), may also constitute an important constraint for nematode abundances at deep-sea cold seeps (Van Gaever et al. 2006, 2009c, Zeppilli et al. 2011).

1.2 Taxon and functional structure

The structure of faunal communities, including those in deep-sea environments, within a given locale is determined by the regional taxon pool, shaped through historical processes, as well as the colonization and dispersal ability of the various taxa, biotic interactions, and taxon-specific tolerances of the prevailing environmental conditions (Ricklefs 1987, McClain and Hardy 2010). One of the most important structuring agents in the deep sea is the deposition of organic matter which varies both spatially and temporally (Lampitt and Antia 1997, Lutz et al. 2007). The longitudinal gradient in surface primary productivity from the GB region towards the more oligotrophic eastern Mediterranean, and hence the deposition of POC on the seafloor, was associated with a graded transition in nematode taxon and trophic composition (**Chapter 4**). This longitudinal gradient in community structure was partly driven by the higher relative abundance of predatory/scavenging nematodes (feeding type 2B), notably *Sphaerolaimus* and *Metasphaerolaimus*, in the eastern part of the transect, even though total nematode standing stock was lower here. We ascribed this to the diminished sedimentation rates in the eastern Mediterranean stimulating the development and establishment of trophic groups that not directly depend on the organic matter flux (Sibuet et al. 1993, Gage 2003). Tselepides et al. (2000) and Kröncke et al. (2003) also observed higher dominance of carnivorous polychaetes at deeper, and thus more oligotrophic, stations in the eastern Mediterranean. Our observation of a higher relative abundance of predatory/scavenging nematodes in the more oligotrophic eastern Mediterranean contrasts with the increased contribution of this feeding guild to communities residing in canyon sediments which receive high, albeit temporally variable, input of relatively labile organic matter (Soetaert and Heip 1995, Ingels et al. 2009). Ingels et al. (2009) attributed the success of the predatory/scavenging nematodes (primarily Sphaerolaimidae as in the eastern Mediterranean) in the Nazaré canyon to their greater size and length, enabling them to

promptly evade the frequent disturbances by gravity flows and intense currents and storms. An analogous explanation for the increased importance of predators/scavengers in the eastern Mediterranean basin may be that they can respond more quickly to the arrival of food because they are longer and heavier and thus more mobile relative to the other nematodes (Soetaert et al. 2002, Schratzberger et al. 2004). Alternatively, the higher proportion of predators/scavengers in some canyons and in the eastern Mediterranean may be caused by the reduced predation pressure (owing to the low standing stock of larger biota), which may select for larger, surface-dwelling meiofauna such as most predatory/scavenging nematodes, or by reduced competition with larger fauna (presuming these do not select for larger predatory or scavenging nematodes) for the deposit-feeding nematofauna. Notwithstanding predatory polychaetes dominate macrofaunal communities in the deep eastern Mediterranean, total macrofaunal biomass is very low in this basin (Tselepides et al. 2000, Kröncke et al. 2003). Omnivorous or predatory macrofaunal species are poorly represented in the middle and lower portions of the Nazaré canyon (Cunha et al. 2011), investigated by Ingels et al. (2009). However, diminished competition or predation is merely a hypothesis and remains to be verified. In the deep sea, the mean and variance of productivity are generally positively related (Rex and Etter 2010). This positive association was, however, not true for our stations sampled. The GB region, which was assigned maximal POC flux estimates according to the model of Lutz et al. (2007), displayed maximal seasonal variability in primary productivity, and so we assumed, in seafloor POC flux. In contrast, in the Mediterranean Sea, the western basins received a higher, but less variable POC flux compared to the eastern basins. The lower nematode maturity index in regions with higher seasonal variability in primary productivity suggests that deep-sea nematode communities experience a more pulsed nutrient loading as a disturbance (**Chapter 5**). Despite the west-to-east cline in community composition, the nematode assemblages inhabiting the slope sediments sampled at the GB region and the Mediterranean were all dominated by genera like *Acantholaimus*, *Halalaimus* and *Thalassomonhystera*, which prevail at slope locations worldwide (Vanreusel et al. 2010) (**Chapters 3 and 4**).

The station atop of the GB, the so-called seamount station, was characterized by a distinct nematode community with a relatively large share of *Desmodora*, *Bolbolaimus* and *Pselionema*. These or closely related genera were also reported as predominant at the Great Meteor and Sedlo seamounts, which are situated south of the Azores (and the GB) in the northeast Atlantic (Vanreusel et al. 2010b). These assemblages may be structured by the coarser texture of the sediments (Vanreusel et al. 2010b). However, unlike these seamounts (Gad 2009, Vanreusel et al. 2010b), the GB also harboured a considerable number of typical slope nematode genera like *Acantholaimus* and *Daptonema*, while Draconematidae and Epsilonematidae were absent and scant, respectively. The seamount assemblage contained a relatively large proportion of

epistrate feeders (feeding type 2A), which was construed as an adaptation to the coarse sediments, from which they can scrape off the microbial coating using their tooth or teeth (Moens and Vincx 1997).

Cold seep sediments display high spatial heterogeneity in pore-water concentrations of methane, sulphide, and oxygen as well as in seepage intensity, and this environmental mosaic shapes the composition of seep fauna (Olu et al. 1997, 2004, 2009, Tryon and Brown 2001, Levin et al. 2003, Van Dover et al. 2003, Menot et al. 2010a). The different sites sampled along the 10 m transect at the Darwin MV showed a high variability in upward methane flow, which peaked at the seep site and ceased at a 5 m distance (**Chapter 2**). Accordingly, nematode genus composition differed between sediments showing signs of methane seepage and those that did not. *Sabatieria* and *Desmodora* were the predominant nematode genera at the Darwin MV seep site. One or both of these genera also prevailed at the REGAB pockmark in the southern northeast Atlantic (3151 m) (Van Gaever et al. 2009a), at shallow (141-163 m) mud volcanoes in the Sicily channel (Zeppilli et al. 2011) and in the deep eastern Mediterranean (A. De Groote, pers. comm.). The *Sabatieria* specimens at the REGAB seep and at the eastern Mediterranean mud volcanoes were identified as *S. mortenseni*, whereas at the Darwin MV seep site the most abundant species were *S. vasicola* and *S. punctata*. All nematode species documented to attain high abundances in seep sediments are widespread and also thrive in organically enriched shallow marine sediments (Van Gaever et al. 2009c, Vanreusel et al. 2010a). Hence, cold seeps may have been colonised by opportunistic shallow-water species, or the other way around, owing to the comparable, favourable environmental regime. Oceanic currents (e.g. Mediterranean Outflow Water), and the seaweeds (Arroyo et al. 2006) or organic particles (Shanks and Walters 1997) they carry, may have been responsible for the exchange of species between the shallow and the deep marine realm. Molecular analysis can aid in resolving phylogeographic patterns within the deep sea (Derycke et al. 2008, Bik et al. 2010). Nevertheless, the question remains whether shallow and deep-sea cold seep species actually constitute the same species, since genetic studies have revealed the existence of several marine cryptic species complexes (Bhadury et al. 2008, Derycke et al. 2008). *Halomonhystera disjuncta*, the dominant species within microbial mats at the Håkon Mosby MV, was pinpointed as one of the five cryptic species within the *H. disjuncta* species complex (Derycke et al. 2007, Van Gaever et al. 2009c).

Despite the outspoken dominance of *Sabatieria* and *Desmodora* at the Darwin MV seep site, genus composition changed only gradually with increasing distance from the seep. This graded transition in community structure can be regarded as a continuous succession of genera along a gradient of organic enrichment (Pearson and Rosenberg 1978). Nevertheless, *Sabatieria* was

recorded as a (sub)dominant genus in all sediment cores collected. This finding may be related to the small surface area of the seep sediments (100 cm^2), which may experience a relatively intense interchange of nematode species with the surrounding sediments. In agreement, Van Gaever et al. (2010) showed that the nematode community composition of small bacterial patches was more similar to their surroundings in comparison with larger bacterial mats at the Håkon Mosby MV. Thus, some stress-tolerant nematodes from neighbouring, less sulphidic sediment patches at the Darwin MV may migrate into the seep site to feed, but when anoxia or the prolonged exposure to hydrogen sulphide starts to impair their performance, nematodes leave again to recover.

Sabatieria sampled at the Darwin MV were identified down to species level (**Chapter 2**, Table 4). None of these species were restricted to the seep sediments, and all species occurred in seep-influenced and non-seep sediments. Nonetheless, *S. vasicola* and *S. punctata* dominated at the seep site, whereas their share in total nematode abundance farther from the seep site was considerably lower. The differing relative abundances of the *Sabatieria* species at the Darwin MV sites illustrates that species belonging to the same genus can respond differently to environmental conditions. This implicates that genera cannot be utilised as a proxy for species at the Darwin MV, as was concluded for the Kenyan continental margin based on distinct spatial patterns in species and genus diversity (Muthumbi et al. 2011). In contrast, Leduc et al. (2012a) found that turnover diversity of nematode genera was driven by the same environmental parameters as species turnover diversity. We hypothesize that data at the genus level can be used for the evaluation of macro-ecological trends, but that species abundances provides more detailed information on processes operating at the local scale (e.g. competition, predation, etc.).

1.3 Taxon and functional diversity

Several authors have acknowledged the high taxon diversity of nematodes in the deep sea (Soetaert et al. 1991, Mokievsky and Azovsky 2002, Lambshead and Boucher 2003, Danovaro et al. 2009a). The stations in the GB region and in the Mediterranean Sea were characterized by a high number of nematode genera (EG(20) = 11.02 - 14.63) with no outspoken dominance (J' = 0.76 - 0.95) (**Chapter 5**). Food availability, here determined as the annual amount of seafloor POC flux, not only influenced standing stock and community structure of the nematodes (**Chapter 4**), but also impacted taxon diversity (**Chapter 5**). Our results further indicated that the magnitude of and variability in seafloor POC flux may influence the phylogenetic diversity (measured as taxonomic distinctness) of nematode communities (**Chapter 5**). Given the variety of factors (i.e. biogeography, habitat characteristics, environmental variability, stress...) that may

influence average taxonomic distinctness within communities (e.g. Warwick and Clarke 1995, 1998, Mouillot et al. 2005, Leira et al. 2009, Xu et al. 2011, Bevilacqua et al. 2012), we can only speculate on the mechanisms that steer the observed patterns. Hence, our results imply that both the magnitude of and the variability in seafloor POC flux were partly responsible for the longitudinal and bathymetric trends in nematode diversity along the transect between the Galicia Bank and the more oligotrophic Levantine basin. Note, however, that regular sampling of deep-sea nematodes from the same locale would undoubtedly allow for a better assessment of the impact of variable POC input on nematode diversity.

Sediment particle size diversity is believed to promote nematode diversity through increased habitat heterogeneity (through the influence of interstitial space on body dimensions, e.g. Tita et al. 1999) or resource partitioning (through the relation between feeding efficiency and grain size, e.g. Gallucci et al. 2005). We found no relationship between sediment particle size diversity and nematode genus diversity in contrast to Leduc et al. (2011) (positive relationship) and Netto et al. (2005) (negative relationship). However, comparison between these previous (Leduc et al. 2011: 5 size classes; Netto et al. 2005: 23 size classes) and present (10 size classes) observations is hampered by the different size classes used to calculate the sediment diversity index. Vanaverbeke et al. (2002) demonstrated that nematodes in subtidal sandbanks are sensitive to minute differences in sediment composition, and thus the number and delineation of the size fractions used to compute sediment heterogeneity may be a crucial factor. We did, however, observe elevated taxonomic distinctness in more heterogeneous sediments. A greater variety of particle sizes may reflect a greater variety of niches, which may be occupied by taxonomically, and potentially functionally, more dissimilar nematode species. In the study of Leduc et al. (2011), the higher genus diversity may have been associated with a higher taxonomic distinctness. Etter and Grassle (1992) and Levin et al. (2001) speculated that the positive relation between sediment and macrofaunal species diversity may be the consequence of the actions of more diverse assemblages generating higher particle size diversity, and not the other way around. Given their small size, this mechanism appears rather unlikely for deep-sea nematofauna. Experiments either controlling for sediment heterogeneity (for instance, by checking nematode colonization and succession of multiple defaunated sediment trays with differing particle size diversity) or nematode diversity (for example, by lowering oxygen levels and therefore diversity, and by tracking changes in sediment heterogeneity) may shed light on the mechanism behind the presumed link between nematode and grain size diversity.

Opposed to the photosynthetically driven control sediments near the Darwin MV, the seep site was characterized by low taxon diversity (**Chapter 2**), in agreement with several other studies on seep nematofauna (Shirayama and Ohta 1990, Van Gaever et al. 2006, 2009a, c, Guilini et al.

2012). The low diversity is the consequence of the harsh environmental conditions which can only be tolerated by a limited set of opportunistic genera (here mainly *Sabatieria* and *Desmodora*). These genera are then able to flourish owing to the reduced competition and predation stress. Tolerance of and proliferation under these low/zero oxygen levels and high sulphide concentrations implicates some sort of behavioural, morphological or physiological adaptation. Scanning Transmission Electron Microscopy Energy Dispersive X-ray analysis or STEM-EDX analysis showed empty vesicles, which possibly contained elemental sulphur prior to ethanol dehydration, in the cuticle of *Desmodora* specimens inhabiting the Darwin MV seep sediments. Similar structures were observed in thiobiotic nematodes from shallow hydrothermal vents (Thiermann et al. 2000) and mangrove flats (Nicholas et al. 1987) as well as in free-living and symbiotic sulphur-oxidizing bacteria (Pasteris et al. 2001, Himmel et al. 2009, Maurin et al. 2010). In contrast to hydrogen sulphide, elemental sulphur is more benign, and upon re-entry in oxygenated sediments, it can be oxidized to non-toxic water-soluble sulphur compounds and removed from the body (Powell et al. 1979). In future investigations of sulphide detoxification mechanisms in seep nematodes, Raman microspectrometry should be applied as it allows for a quick investigation of living organisms and it does not require any (sulphur-dissolving) sample pre-treatment (Pasteris et al. 2001, Maurin et al. 2010). Contrary to *Desmodora*, the genus *Sabatieria* did not show any detoxification structures or symbiotic bacteria. We presumed a morphological and consequently behavioural adaptation whereby the great length of the dominant *Sabatieria* species (*S. vasicola* and *S. punctata*, also applicable to the long *Desmodora*) promotes rapid migration between toxic but food-rich and non-toxic, food-poor sediment patches. In addition to enhanced mobility and vesicles of elemental sulphur, the genera thriving at the Darwin MV seep site might be able to switch to sulphide-insensitive, anaerobic metabolism when foraging in seep sediments (McMullin et al. 2000). Biochemical analysis of seep nematodes would answer our question regarding how nematodes are able to survive in these sulphidic environments.

With increasing distance from the seep site, nematode assemblages at the Darwin MV had higher taxon and trophic diversity. This pattern conforms with the model of Pearson and Rosenberg (1978) describing the succession of taxa along a gradient of organic enrichment. The Darwin MV seep site represents the source of organic matter enrichment where few opportunistic taxa can survive. Farther away from the organic carbon source, environmental conditions are less deleterious and the ample food attracts many taxa that are sensitive to sulphide and/or oxygen stress next to the opportunistic taxa already present. The elevated trophic diversity reflects the presence of both thiotrophic and phototrophic carbon sources allowing for greater resource partitioning compared to the seep sediments.

2 Biological drivers of deep-sea nematode community attributes

Not only purely environmental agents but also other biota, besides those that may represent food for deep-sea nematodes, probably influence the distribution, structure and diversity of deep-sea nematode assemblages. In coastal habitats, macro- and megafauna are regularly observed to depress nematode standing stock, presumably by means of physical disturbance or (unintentional) predation (Kennedy 1993, Schratzberger and Warwick 1999, Tita et al. 2000, Danovaro et al. 2007). Subsurface density peaks observed for the nematofauna result from predation, competition, or physical disturbance at the sediment surface, or from stimulation of bacterial production or oxygenation deeper in the sediment by macrofauna (e.g. Braeckman et al. 2011). In addition, larger faunal groups have been shown to affect species diversity, as well as trophic and species composition of subtidal, intertidal and estuarine nematode assemblages (Schratzberger and Warwick 1999, Tita et al. 2000, Braeckman et al. 2011).

In deep-sea environments, there have been very few investigations into the interaction between nematodes and other benthic size groups. At the North Carolina continental slope, tube-building polychaetes were observed to draw down freshly deposited organic matter which was fed upon by deep-dwelling nematodes (Levin et al. 1997). Gallucci et al. (2008) found elevated densities, but reduced diversity of nematodes upon exclusion of the megafauna at the Arctic Hausgarten site. Instead of direct investigations, meiofauna-macrofauna or meiofauna-megafauna interactions have been mostly inferred from spatial distribution patterns. Negative correlations between meio- and macrofaunal abundances were considered an indication of predation or competition (Debenham et al. 2004, Van Gaever et al. 2009c). Subsurface nematode density maxima in the Molloy Deep were ascribed to the dense holothurian population (Soltwedel et al. 2003). There have been several studies though, addressing the influence of biogenic structures on nematode distribution, diversity and community structure (e.g. Van Gaever et al. 2004, Raes and Vanreusel 2006, Hasemann and Soltwedel 2011). Clearly, there is a huge gap in our understanding of the interaction between nematodes and other faunal groups in the deep sea and more experimental and integrative observational studies are definitely needed.

3 Ecosystem function of deep-sea nematodes

Because nematodes are heterotrophic organisms and thus need to consume organic substrates for their energy and carbon requirements, they directly contribute to the cycling of organic matter. In addition to their feeding behaviour (discussed in Section 3.1), other ecological aspects (Section 3.2) may promote ecosystem properties and processes.

3.1 Feeding ecology

From the morphology of the mouthparts, Wieser (1953) deduced feeding strategy and the concomitant range of possible food items for free-living marine nematodes. This author then developed a classification system according to which nematode genera could be assigned to one of four feeding guilds based on the armature and size of the buccal cavity (Chapter 1), i.e. selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate feeders (2A) and predators/scavengers (2B). Observational studies confirmed the purported link between buccal morphology and feeding ecology, though several modifications were proposed (e.g. splitting group 2B into facultative and obligate predators, Moens and Vincx 1997; lumping selective and non-selective deposit feeders, Jensen 1987a; usage of cephalic setae in foraging, Romeyn and Bouwman 1983); some of which were rejected later on (removal of the subdivision between selective and non-selective deposit feeders, Thistle et al. 1995, Moens and Vincx 1997; usage of cephalic setation in foraging, Jensen 1987a, Moens and Vincx 1997). However, both observations and experiments involving labeled food items indicated that nematodes with similar mouth morphologies were in fact feeding dissimilarly (Moens et al. 1999a, b, Olafsson et al. 1999). Furthermore, Wieser's trophic classification does not account for feeding plasticity (Moens et al. 1999c, Fonseca and Gallucci 2008) and only considers particulate matter as food for marine nematodes, although lab experiments evidenced the ability of shallow-water nematodes to drink or absorb dissolved organic substances (Chia and Warwick 1969, Lopez et al. 1979, Riemann et al. 1990, Moens et al. 1999c). Despite these shortcomings, the majority of current nematode ecological studies in shallow (e.g. Danovaro and Gambi 2002, Vanaverbeke et al. 2004, 2011) and deep (e.g. Gambi et al. 2003, Fonseca and Soltwedel 2007, Ingels et al. 2009) marine habitats still uses the original scheme of Wieser (1953).

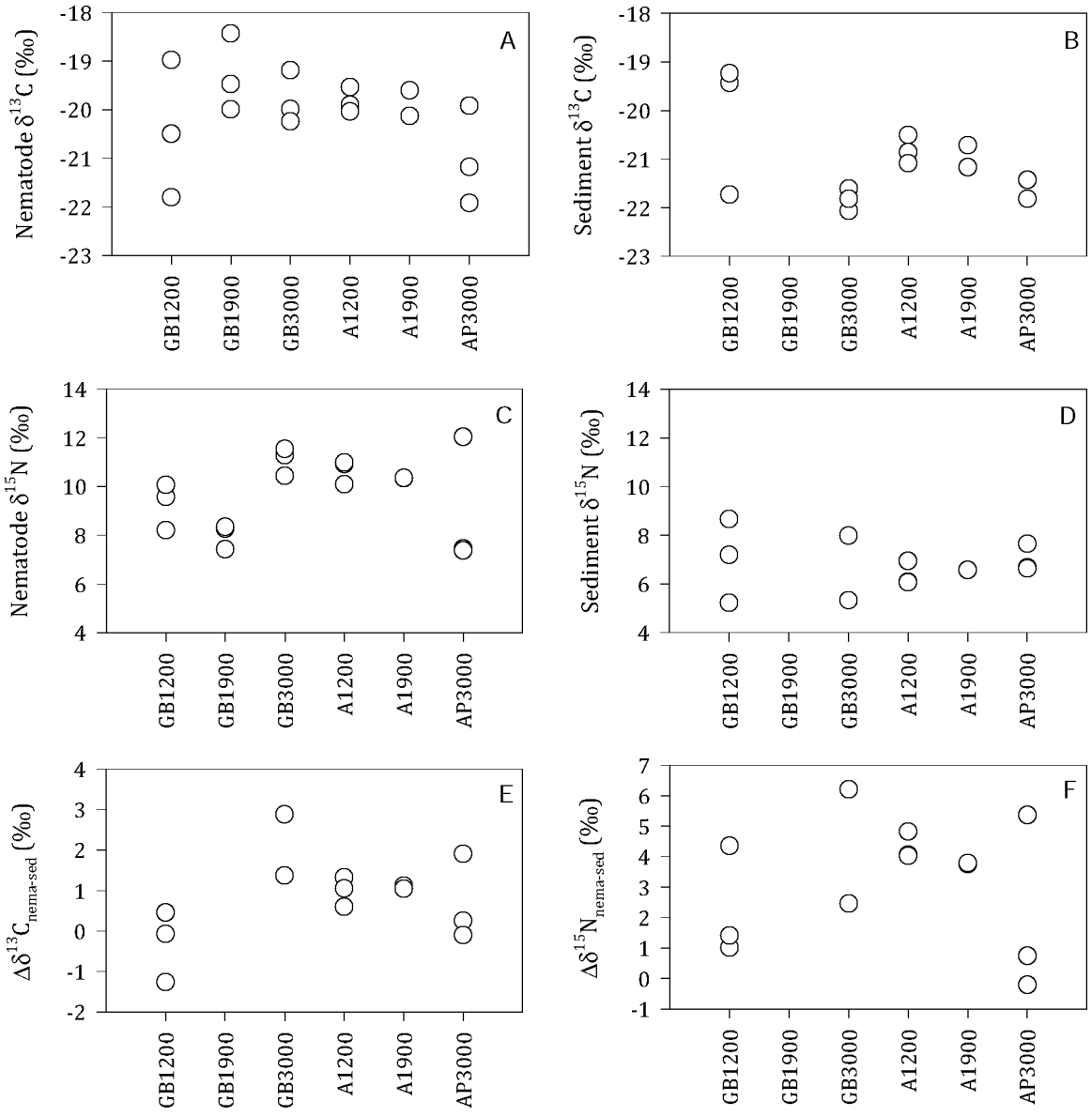

Fig. 1. *Natural carbon and nitrogen stable isotope signatures of nematodes (A and C) and sediment organic matter (B and D), and the isotopic shift between nematodes (nem) and sediment organic matter (sed) (E and F) per station. Station codes indicate basin of origin (GB: Galicia Bank region, A: Algerian basin, AP: Algero-Provençal basin) and approximate water depth (1200, 1900, or 3000 m).*

The natural stable isotope composition of an animal's body tissue can serve as a useful tool to decipher its nutrition and trophic position. In general, the carbon isotope signature ($\delta^{13}C$) of an organism reflects that of the diet within 1 ‰ (Michener and Kaufman 2007). The nitrogen isotope signal ($\delta^{15}N$) can be used to indicate the trophic level of a consumer since, on average, there is an enrichment of 3.4 ‰ relative to its diet (Post 2002). However, these commonly utilized average trophic shift values should be applied with caution. Several studies have shown considerable variation in the degree of fractionation of nitrogen, and to a lesser degree of

carbon, dependent on, amongst others, the taxonomic identity, the dietary composition and the nutritional status of the consumer (e.g. McCutchan et al. 2003, Vanderklift and Ponsard 2003, Caut et al. 2009). Even when the trophic fractionation is known, the exact dietary composition can only be precisely determined using the stable isotope approach when the isotope values of all potential food sources are known and these do not display high overlap. In trophic ecology studies, however, these conditions are rarely fulfilled. Isotope mixing models can offer a range of possible solutions for underdetermined mixing problems, but these assume that all sources sampled contribute to the consumer's diet (Fry 2013). We applied dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) to bulk nematode samples from the GB region and the western Mediterranean (Algerian and Algero-Provençal basin) with the intention of comparing these with fatty acid profiles. Since fatty acid analysis was unsuccessful (see further) and these data did not add much to the results in Chapter 3, these were not included therein. Both carbon and nitrogen stable isotopes of sediments and nematodes are presented in Fig. 1. Nematode carbon isotope values (-21.9 to -18.4 ‰,) were in the same range as those of the sediment pool (-22.1 to -19.1 ‰), indicative of bulk sedimentary organic matter as the main nematode food source. The difference in nitrogen isotope signatures between nematodes (7.4 - 12.0 ‰) and sediments (5.2 - 8.7 ‰) ranged between -0.2 and 6.2 ‰. Given the high variability in nitrogen trophic fractionation (see above), nematodes may be feeding on the bulk sedimentary organic matter directly (which is largely semi-labile or refractory in nature), or indirectly via, for instance, microbial prokaryotes or eukaryotes (cfr. Iken et al. 2001).

In our study addressing the feeding ecology of seep nematodes (**Chapter 2**), the two predominant genera, *Sabatieria* and *Desmodora*, and the rest of the nematodes sampled were analyzed separately for carbon and nitrogen stable isotope composition. The light carbon isotope values (nearly all $\delta^{13}C$ values fell below -28 ‰) indicated that nematodes relied upon thiotrophic carbon up to 10 m away from the seep site. This thiotrophic carbon originated from sulphur-oxidizing bacteria, which were either directly preyed upon by the nematodes or were assimilated indirectly via the drinking/absorbing of bacterial lysates or via the consumption of bacterivores, such as heterotrophic bacteria or protists. In support of this latter nematode feeding scenario, Olafsson et al. (1999) noted that freshly fixed *Sabatieria pulchra* regurgitated ciliates upon placement in brackish water. The nematodes collected at and near the Darwin MV seep site were not particularly slender, which has previously been regarded as an adaptation in thiobiotic nematodes to facilitate uptake of dissolved substances through the body wall (Jensen 1986, 1987b). However, epidermal absorption of dissolved organic matter may not be restricted to slender specimens only (Chia and Warwick 1969, Lopez et al. 1979, Riemann et al. 1990). By using instrumentation adapted for small samples, Carman and Fry (2002) obtained stable isotope values for two marsh nematode species. Given the low biomass of deep-sea nematodes,

this technique can generally not be applied at the species or genus level in the deep sea realm. However, new technology such as a nanoSIMS (brand name for high spatial resolution dynamic secondary ion mass spectrometry) instrument allows for, amongst others, the accurate measurement of isotope ratios at a high spatial resolution (50-100 nm) (review by Boxer et al. 2009). Hence, this technology can be applied for the analysis of the bulk tissue stable isotope composition of individual deep-sea nematodes. Naturally, individual stable isotope values will provide a more accurate picture of nematode trophic interactions. Isotope signatures at such high resolution can be used to compute a set of trophic diversity metrics (Layman et al. 2007), which can subsequently be compared with the traditional measure of trophic diversity based on mouth morphology (Heip et al. 1985).

Fatty acids are an essential element of neutral lipids, i.e. triglycerides and wax esters, which serve as an energy storage, and of phospholipids which form biological membranes. Some (combinations of) fatty acids are unique to particular taxa and can be used to reveal the dietary composition of organisms, assuming the assimilated fatty acids are left relatively unmodified (Dalsgaard et al. 2003, Kelly and Scheibling 2012). Marine microalgae are rich in FAs with more than two double bonds, i.e. polyunsaturated fatty acids (PUFAs), notably eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n-3) acid. Bacteria chiefly incorporate fatty acids in their phospholipids, which are generally odd-numbered, saturated (no double bonds) or mono-unsaturated (one double bond) and often contain a methyl-branched chain (Boschker and Middelburg 2002, Berge and Barnathan 2005). Also PUFAs have been detected in deep-sea microbial populations (Nichols and McMeekin 2002, Nichols 2003), but the scope of the capacity of PUFA synthesis among deep-sea bacteria is unknown. Compared with stable isotope composition, fatty acid profiles provide more detailed information on the food organisms (since these can indicate the specific group of algae or bacteria consumed) and may evidence bacterial feeding. Unfortunately, fatty acid analysis requires an even greater amount of carbon than dual stable isotope analysis, which is often difficult to realise for deep-sea nematodes. Fatty acid signatures were successfully assessed for nematodes from the intertidal (Leduc 2008), a deep cold seep (Van Gaever et al. 2009b), and the Antarctic deep sea (Guilini et al. 2013). Owing to the low densities and small size of nematodes from the deep Mediterranean basin, obtaining sufficient biomass for fatty acid samples was cumbersome. Where we succeeded in acquiring a sufficiently large sample, results were generally unreliable (owing to unexpectedly low concentrations of standard FAs or "tailing" in the chromatograms, the latter pointing to contamination). Therefore, we decided to leave this research path, at least for the present doctoral research. The most reliable chromatogram that we obtained is shown in Fig. 2. This graph shows the presence of both bacterial and micro-algal markers, in addition to several fatty acids which are encountered in multiple organic matter sources.

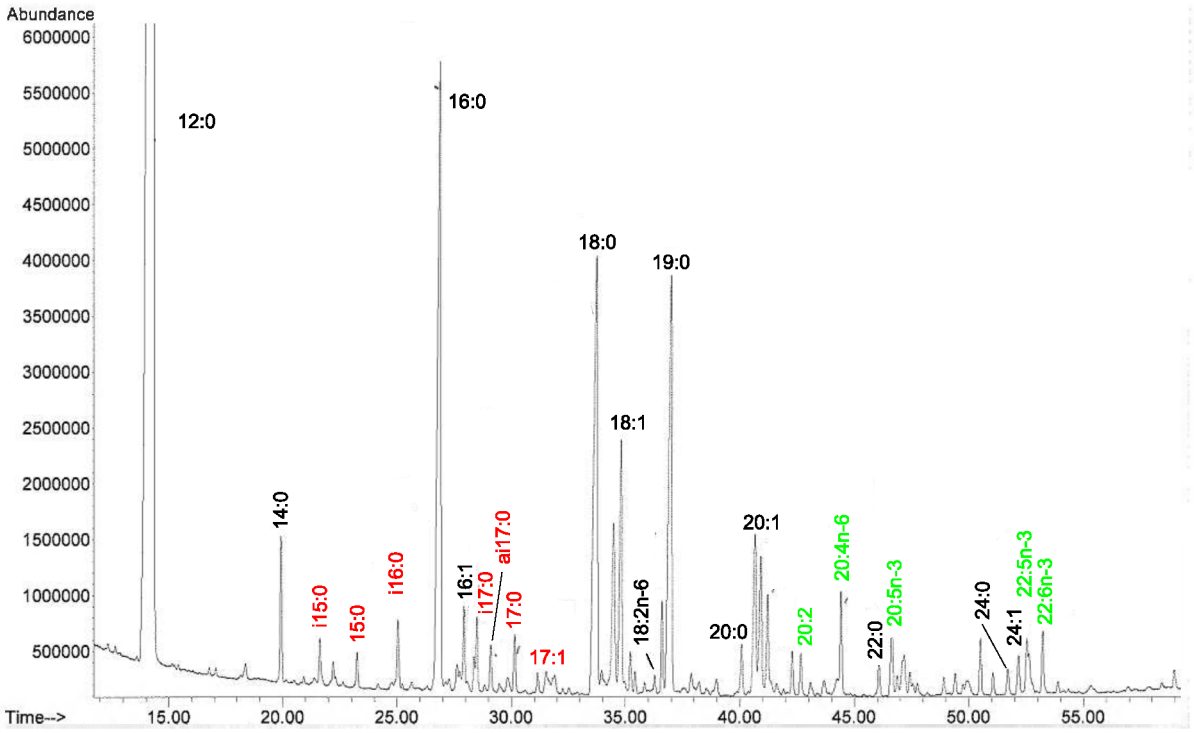

Fig. 2. *Chromatogram showing concentrations of fatty acids in a bulk nematode sample. This sample contained 994 nematodes extracted from sediments from 1200 m depth in the western Mediterranean. Fatty acids are denoted as A:Bn-x, where A is the number of carbon atoms, B is the number of double bonds, and x is the position of the first double bond relative to the terminal methyl group. Bacterial markers are shown in red, while micro-algal markers are indicated in green. The remaining fatty acids (in black) are not unique to bacteria or algae.*

By administering carbon stable isotope labelled food items to marine sediments, it is possible to follow up the carbon processing dynamics within benthic environments. We conducted experiments using ^{13}C enriched dissolved organic matter (DOM) in the GB region and the western Mediterranean (**Chapter 3**), aimed at labelling the indigenous heterotrophic bacteria and the nematodes, the latter through bacterivory or direct DOM consumption. The time-series experiment at the station atop of the GB seamount (GB1200) was the first (compare with Guilini et al. (2010) for Arctic deep-sea sediments, and with van Oevelen et al. (2006a) for intertidal sediments) to reveal a higher ^{13}C enrichment of nematodes than both sedimentary organic matter and bacteria. The seamount station also displayed elevated nematode assimilation rates compared to the deeper slope stations. The unprecedented high nematode label uptake at the seamount station was ascribed to the aberrant environmental conditions (relatively strong hydrodynamic regime) and the resultant nematode community structure (several of the

predominant genera are atypical of deep-sea sediments), and may represent an adaptation to a food-poor environment. Low food availability at the seamount station was inferred from the low bacterial biomass, and the low sedimentary concentrations of organic matter and nitrogen. The decomposition rate of organic matter in permeable shelf sands can exceed that in fine-grained sediments because of the differential pore-water transport modes (i.e. advection vs. diffusion, respectively) (Franke et al. 2006, Rusch et al. 2006). Advective pore-water flow may stimulate organic matter breakdown through the transport of nutrients, electron acceptors and organic matter into the sediment, and of inhibitory metabolites out of the sediment, as well as through the spatial decoupling of particulate and final dissolved organic matter decomposition (Franke et al. 2006). Hence, under *in situ* conditions, the discrepancy in nematode carbon assimilation between the seamount and the slope stations may have been even higher. Nevertheless, total carbon assimilation was minimal at the seamount station owing to the low biomass of benthic bacteria, which dominated the biologically mediated flow of DOM carbon, also at the deeper slope stations. Nematodes contributed only very little (max. 2.4 % of the total amount processed by bacteria and nematodes together) to the processing of dissolved organic matter.

Another conclusion drawn on the basis of the empirical results was, as suggested earlier in this general discussion, that the trophic classification scheme of Wieser (1953) cannot always be used to make quantitative statements about the dietary composition of marine nematodes. Communities with similar buccal morphologies showed distinct assimilation rates (i.e. stations GB1200 and GB1900, **Chapter 3**), whereas assemblages differing in trophic structure took up similar amounts of the ^{13}C label (stations WMed1900 and GB1900, **Chapter 3**). Inconsistencies between food uptake in an experimental setting and the categorization based on buccal morphology were also found by Olafsson et al. (1999) for subtidal sediments and by Guilini et al. (2010) in the Arctic deep sea. Based on nematode weight-specific respiration rates and ^{13}C enrichment, bacteria were construed as a more plausible candidate food source than DOM. Nonetheless, bacterivory was still insufficient in light of the relative ^{13}C enrichment of nematodes and biomass-specific respiration, consistent with the work of Guilini et al. (2010) and Gontikaki et al. (2011a). However, Guilini et al. (2010), Gontikaki et al. (2011a) and we calculated nematode respiration rates following the formula of de Bovée and Labat (1993), which is valid under oxic conditions. Given the recently demonstrated overestimation of nematodes respiration rates calculated following de Bovée and Labat (1993) in sediments that are not fully saturated with oxygen (Braeckman et al. in press), bacterial carbon assimilation may actually fulfil a greater part of nematode carbon demands. Furthermore, the estimates of nematode assimilation efficiency (the amount of carbon ingested that is assimilated/absorbed through the intestinal wall) and net growth efficiency (i.e. the amount of carbon assimilated that is channelled towards production) employed in this (**Chapter 3**) and other work (Soetaert and

van Oevelen 2009, van Oevelen et al. 2011, 2012) are based on findings from lab-cultured shallow-water species (see references in van Oevelen et al. 2006c). In addition, we used a fixed value for assimilation and growth efficiency, and these may well vary according to the quality and quantity of the food offered, as reported for prokaryotes (Del Giorgio and Cole 1998), some freshwater small invertebrates (Jeyasingh 2007), but also for the estuarine nematode *Litoditis marina* (Moens and Vincx 2000; formerly known as *Pellioditis* or *Rhabditis marina,* see Sudhaus 2011). It would be interesting to measure assimilation and growth efficiencies in deep-sea nematodes when offered a variety of potential food sources. Deep-sea nematodes from the GB seamount (1200 m depth) could be kept alive in the lab for at least 4 weeks within the sampled sediment cores (pers. obs.), and also *Halomonhystera disjuncta* from the Håkon Mosby MV (1280 m depth) survived several months on agar plates (J. Van Campenhout, pers. comm.). Regardless of the uncertainties associated with metabolic rates, deep-sea nematodes may not feed extensively on sedimentary bacteria. Other, hitherto ignored, candidate food items for deep-sea nematodes include fungi (suggested by the co-occurrence of nematode and fungal phylotypes in deep-sea nematodes, Bhadury et al. 2011), protists (Hamels et al. 2001) and bryozoans (Lidgard 2008). However, since many protists rely on bacteria for their nutrition (Sherr and Sherr 2002), extensive feeding on this group by the nematodes should probably have resulted in higher nematode ^{13}C assimilation rates. Linear inverse modelling of deep-sea benthic carbon flows pointed at refractory organic matter as the main food source of nematodes (Gontikaki et al. 2011b, van Oevelen et al. 2012). Generally, labile organic matter is presumed to be a more palatable food source because it is decomposed more easily than semi-labile or refractory detritus. If nematodes do feed on less labile organic matter, they may possess specialized digestive enzymes or intestinal bacteria. Feeding experiments using ^{13}C enriched detrital organic matter may confirm the modelled substantial assimilation by deep-sea nematodes.

3.2 Other nematode functions

By engaging in feeding, defecating, respiring and other related processes, free-living nematodes directly contribute to the cycling of organic matter in marine sediments. In addition to the processing of organic carbon, nematodes may stimulate mineralisation of organic matter by small-scale bioturbation and bio–irrigation activities, referred to as cryptic bioturbation and bio-irrigation (Cullen 1973, Pike et al. 2001). Small tunnels and cavities created by nematodes have been observed in both shallow (Cullen 1973, Nehring et al. 1990) and deep (Pike et al. 2001) marine sediments. In an experimental setting, the movements of marine nematodes were shown to enhance fluxes of nutrients and oxygen (Aller and Aller 1992), thereby stimulating the bacterial breakdown of organic matter (Alkemade et al. 1992). Nematodes may also have a

positive influence on the production of bacteria by grazing on them or by providing optimal growth conditions within their mucus trails (Riemann and Helmke 2002, Moens et al. 2005). Furthermore, nematodes may contribute to the cycling of organic matter by serving as a meal for other biota. They may either be actively preyed upon (e.g. by juvenile fish, Coull 1990, 1999; foraminiferans, Dupuy et al. 2010; amphipods, Yu et al. 2003) or ingested (un)selectively by larger deposit feeders (e.g. Gontikaki et al. 2011b). Recently, Leduc et al. (2008) showed that the intertidal predatory nematode *Oncholaimus moanae* was characterized by high levels of highly unsaturated fatty acids, making them a high quality food source (Brett and Müller-Navarra 1997). Nevertheless, few data exist on the importance of nematodes for higher trophic levels, especially in the deep sea. The lack of information is partly caused by the higher digestion rates of nematodes compared to other prey items (Coull 1990, Scholz et al. 1991). Biomarker analyses (stable isotopes and fatty acids) of multiple benthic size classes might elucidate the trophic interactions in which nematodes are involved. This approach was proven successful for soil nematofauna (Ruess et al. 2004).

4 Link between diversity and ecosystem functioning in deep-sea nematode assemblages and the influence of environmental conditions

In **Chapter 1**, we presented a scheme in which we outlined the various presumed feedbacks between environmental conditions, biodiversity, ecosystem functioning and ecosystem services (p 26). In Fig. 3, we illustrate which links were evidenced by the data obtained during this doctoral research. We observed an influence of the environmental regime on nematode taxon composition and diversity, both at the cold seep habitat at the Darwin mud volcano (MV) (**Chapter 2**) and at the photosynthesis-driven stations in the Galicia Bank (GB) region and the Mediterranean (**Chapters 3 and 4**). In this work, we evaluated the composition and diversity of effect (traits that affect ecosystem properties and processes; investigated for all stations) and response (traits that govern the response to environmental variability; not investigated for the Darwin MV) functional traits. Phylogenetic diversity (measured as average taxonomic distinctness and the variability thereof) may reflect both effect and response functional diversity. The prevailing environmental conditions determined effect functional composition (trophic composition) and diversity (trophic diversity and average taxonomic distinctness) of the nematode communities at all stations investigated (**Chapters 3, 4 and 5**). In the photosynthesis-driven habitats in the GB region and the Mediterranean, we also observed an influence of the environmental regime on response functional trait diversity (diversity of life

history strategies or c-p diversity) and composition (maturity index). There was a positive relation between expected genus richness (EG(20)) and the diversity of life history strategies (c-p diversity) for all stations in the GB region and the Mediterranean (**Chapter 5**). If a higher diversity in life history characteristics results in a better resilience and resistance against perturbations, this may lead to more temporally stable ecosystem properties and process rates (Yachi and Loreau 1999, Loreau 2000). However, the colonizer-persister (c-p) classification was originally erected for terrestrial nematodes (Bongers 1990), and subsequently modified for coastal species using ecological information (Bongers et al. 1991). Therefore, investigations into the life history characteristics of deep-sea nematodes are warranted to test the validity of these c-p scores for nematode assemblages in this extreme environment.

We were unable to identify a mechanism through which taxon diversity may promote nematode carbon mineralisation rates, the ecosystem function under study, since none of the functional diversity indices calculated (biomass diversity, c-p diversity, trophic diversity, and several phylogenetic diversity measures) related to both taxon diversity and total respiration rates in nematode communities (**Chapter 5**). Instead, we found a direct positive, linear relation between nematode total respiration and two taxon diversity indices (expected genus richness and Shannon-Wiener genus diversity). The findings from the experiments discussed in **Chapter 3** point at the importance of the environmental context and/or composition of nematode assemblages on nematode carbon processing rates.

There are several possible explanations for the lack of confirmation of our hypothesis that higher taxon diversity is associated with higher functional diversity, which in turn stimulates nematode carbon mineralisation rates. First, the functional diversity metrics employed did not adequately reflect the entire range of nematode traits that determine total respiration. This may be because the functional traits investigated do not influence respiration, or because, for some indices, the delineation of the functional groups was too broad. Nematode taxon diversity may promote carbon mineralisation if more diverse assemblages are responsible for a more complete mineralisation of all carbon sources present through either a greater range of feeding strategies or a greater range of digestive systems. The trophic diversity index may not capture the entire scope of feeding strategies. For coastal nematode species, considerable variability in food uptake within feeding guilds was demonstrated (Olafsson et al. 1999, De Mesel et al. 2006), and also the results of the present doctoral research (**Chapter 3**) show that assemblages with a similar feeding type composition may employ very different feeding strategies. A greater variety in digestive enzymes, associated with higher genus diversity, may also stimulate total carbon mineralisation rates. Phylogenetic diversity can be utilized as a proxy for functional diversity when functionally important traits and biotic interactions have a strong phylogenetic signal

(Srivastava et al. 2012). The lack of a significant relation between phylogenetic diversity (measured here as taxonomic distinctness) and total nematode respiration implies this was not the case for deep-sea nematodes. Second, nematode carbon mineralisation was approximated on the basis of total nematode biomass and temperature and may not truthfully represent *in situ* carbon mineralisation rates. Third, local relations (within each region) between functional diversity and ecosystem functions may exist but are blurred upon the combination of different regions because of the across-site environmental heterogeneity (Loreau 2000, Hiddink et al. 2009). Future deep-sea meiofauna research should preferably be conducted in a wide range of environmental contexts to evaluate abiotic influences on the diversity-function relationship. The mechanisms underlying the patterns detected in field observational studies can be deciphered by means of an experimental approach. Thrush and Lohrer (2012) call for an integrative approach where observational studies and experimental work are combined. Finally, the possibility remains that the functional traits of nematodes are not important for carbon mineralisation, and that the significant relationship between taxon diversity and carbon mineralisation is generated through the correlation of both variables with the same environmental factor(s).

The relevance of biodiversity-function research to ecosystem management depends, amongst others, on whether the results from local studies can be translated to regional or global scales (Srivastava and Vellend 2005). Scale-dependency of the relationship between biodiversity and ecosystem functioning has been noted (Bengtsson et al. 2002, Bond and Chase 2002), and may be verified for nematode assemblages in the deep sea by evaluating the link at a single point (one sample), and at local (replicate samples of one habitat combined) and regional scales (multiple habitats combined). Furthermore, organisms possess more than one phenotypical or behavioural trait. The nematode functional properties that were considered here might be important for ecosystem functions other than nematode carbon mineralisation (see Section 3.2). Combining several functional traits into a multivariate index (Walker et al. 1999, Petchey and Gaston 2002, Weiher 2011) may generate a stronger link between taxon and functional diversity (Hasemann and Soltwedel 2011), as well as between functional diversity and the ecosystem function under study. Since ecosystem functioning depends on the various ecosystem functions carried out, the same argument can be made for examining multiple functions (Gamfeldt and Hillebrand 2008, Gamfeldt et al. 2008).

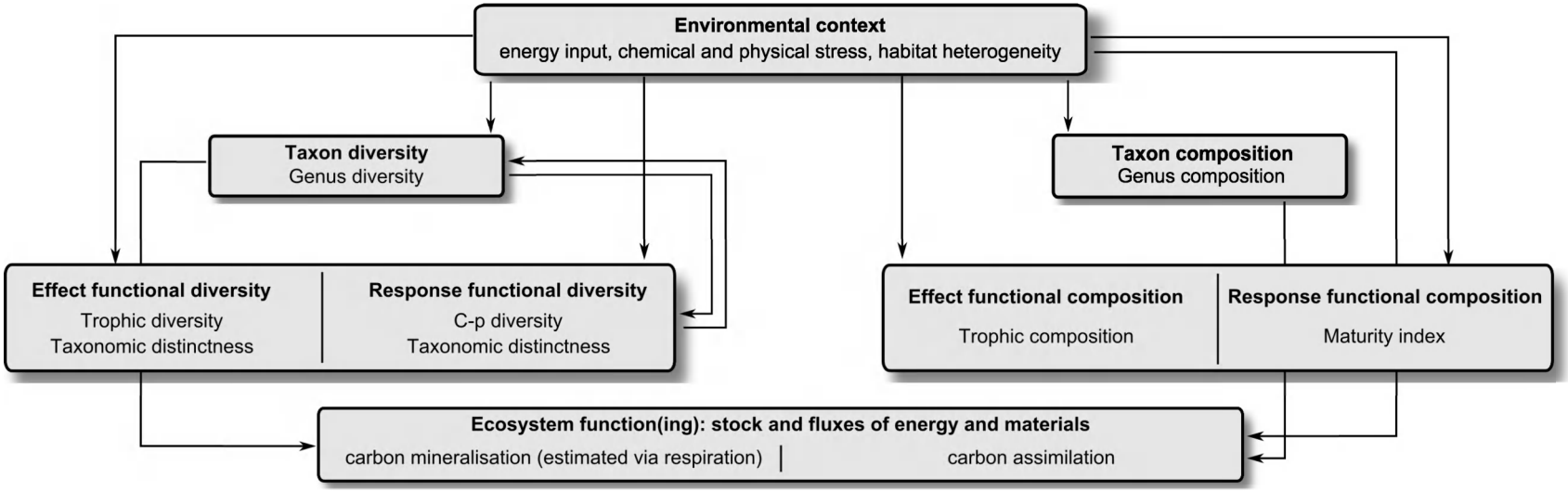

Fig. 3. *Diagram illustrating the links between the environmental context, taxon diversity and composition, (effect and response) functional diversity and composition, and ecosystem functioning which were established for the deep-sea nematode assemblages examined in this doctoral study. C-p diversity: diversity of c-p (life history) classes.*

5 Lessons learnt and room for improvement

In recent years, several studies have reported on the adversary effects of human interference in marine ecosystems. Even bathyal and abyssal macro- and megafauna were demonstrated to be affected by climatic phenomena and the concomitant alteration of the organic carbon flux to the deep seabed (Ruhl and Smith 2004, Ruhl et al. 2008, Smith et al. 2009, Ramirez-Llodra et al. 2011). Our results demonstrate that even the inconspicuous meiofauna, dominated by the nematodes, are under the influence of large-scale upper-ocean and atmospheric processes (i.e. primary productivity and the resultant rain of organic particles to the seafloor). Consequently, the already ongoing and rapidly progressing climate change is expected to exert an influence on the composition, diversity and functions carried out by these small roundworms in the deep-sea benthic environment.

Additionally, the present research identified several caveats within our comprehension of deep-sea nematode ecology, which need to be resolved in the future:

- Despite the extensive change in environmental characteristics with water depth, functions and biological rates observed for shallow marine nematodes are still being extrapolated to congeners thriving in deep-sea sediments. There is a serious lack of laboratory and *in situ* experiments and observational studies on the biology of deep-sea nematodes. Furthermore, the advent of high-resolution technology (for instance, Raman microspectrometry or nanoSIMS analysis) may be used, whether or not in the frame of a collaborative research project, to tackle these issues.

- Nematode species respond differently to local biotic (predation or competition) and abiotic (anoxia or hydrodynamic stress) stressors. Understanding patterns and effects of local diversity requires the identification down to species level, which is, however, especially in the speciose deep-sea environment, an enormously time-consuming but also subjective process. We propose that molecular techniques may be used in the future for a more accurate and presumably faster assessment of local deep-sea nematode diversity.

- Studies addressing the interactions between nematodes and other biota in the deep-sea realm are few. We call for more integrative observational studies to describe spatial and temporal distribution patterns of all (or as many as possible) different components of the deep-sea ecosystem. The existence and nature of biotic interactions, hypothesized on the basis of co-occurrences, may subsequently be verified by experimentation. Collaborative and multidisciplinary projects like BIOFUN and HERMES/HERMIONE provide an excellent framework to conduct such high-resolution research. Within the frame of these projects, integrative papers combining the observations on different taxonomic and/or size components of the deep marine ecosystem are currently in progress (e.g. Ramirez-Llodra et al. in prep., Vanreusel et al. in prep.).

Finally, we would like to stress the importance of employing standardized equipment (surface area of coring devices) and obtaining sufficient replicate samples (to be determined *a priori* by power analysis) in the deep sea to enable a robust statistical analysis of the data obtained.

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