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by Katja Guilini

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The Role of Trophic and Oceanographic Conditions in the Ecology of Deep-Sea Nematodes

De Rol van Trofische en Oceanografische Condities in de Ecologie van Diepzeenematoden

by Katja Guilini

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Katja,

December 2011

Summary

Although the comprehension of deep-sea life is still in its infancy, there is increasing evidence that the deep sea comprises a wide diversity of geological, oceanographic and ecological settings. The highly heterogeneous nature of deep-sea habitats lies at the base of the overall high diversity of benthic organisms found there. Mechanisms that underlie species coexistence on regional and local scale are, however, poorly understood. This inhibits predictions of the effect of increasing human induced impacts on both biodiversity and deep-sea ecosystem functioning in general. Although small-scale processes (e.g. spatio-temporal heterogeneity and disturbance) and small-scale patchiness in species distribution have been the basis for hypotheses on mechanisms as such, limited empirical support exists. Nematodes are the most abundant and species-rich metazoans at small (cm) and intermediate (m) local spatial scales in deep-sea sediments. Together with their ubiquitous presence in deep-sea sediments, this makes them by far the taxon of choice to study the mechanisms that establish and maintain different diversity patterns among deep-sea ecosystems.

Because of their numerical success and standing stocks that are generally proportional to food availability, meiofauna, and in particular nematodes, are assumed to represent a significant component of deep-sea food webs. Nevertheless, unraveling their trophic interactions and functioning in deep-sea sediments has been arduous and challenging. The short-term reaction (max. 23 days) of nematodes to both natural and experimentally simulated depositions of phytodetritus is only minimal or absent compared to bacteria, foraminiferans and several macrofaunal taxa. Therefore to date empirical evidence for deep-sea nematode feeding habits, food preferences, assimilation rates, and in general their significant role in deep-sea environments that depend mostly on the input of phytodetritus, is lacking. This gap of knowledge impedes adequate modeling of deep-sea food webs and bentho-pelagic coupling processes.

The overall aim of this PhD study was to gain insight in the functioning of deep-sea benthic ecosystems. More specific, this study addressed the role of food and oceanographic conditions in the ecology of free-living deep-sea nematodes. Therefore, experiments were performed to gain new insights in the role of bacteria in the diet of nematodes and in the response of nematodes to disturbed, newly available patches, enriched with organic matter (**Chapter 2 and 3**). Field observations from both photosynthetic and chemosynthetic driven ecosystems further addressed the role of both bacteria and phytodetritus in the diet of deep-sea nematodes and had as goal to provide insights in the influence of oceanographic conditions on both the structure and function of nematode communities (**Chapter 4 and 5**).

The broader framework and key features of this thesis are explained and introduced in **Chapter 1**.

Chapter 2 presents a ^{13}C stable isotope labeling experiment that was performed in order to

quantify the importance of bacterial carbon as food source for an Arctic deep-sea nematode community. Instead of treating the bacteria as one black box, several bacterial functional groups were isotopically enriched with different ^{13}C-labeled substrates that were injected into sediments collected from 1280 m depth at HAUSGARTEN (79° N, 6° E, west of Svalbard, ARK XXII-1c, 2007). Incorporation of the ^{13}C label into bacterial phospholipid-derived fatty acids (PLFAs) and nematodes in the top 5 cm of the sediment was monitored over a 7-day period. The ^{13}C dynamics of nematodes were fitted with a simple isotope turnover model to derive the importance of the different bacterial functional groups as carbon source for the nematodes. The different substrates clearly labeled different bacterial groups as evidenced by differential labeling of the PLFA pattern. The deep-sea nematode community however, only incorporated a very limited amount of the label and the isotope turnover model clearly showed that the dynamics of the isotope transfer could not be attributed to bacterivory. Moreover, the low enrichment of nematodes suggests a limited passive uptake of injected ^{13}C labeled substrates. We concluded that dissolved organic carbon compounds directly or indirectly via bacteria are not important as carbon sources for these deep-sea nematodes.

The study presented in **Chapter 3** tries to elucidate the importance of nematode infaunal migration in determining small-scale temporal and spatial heterogeneity and the role of organic matter deposits in this process in the deep sea. Therefore a colonisation experiment was performed in situ at 2500 m water depth at the Arctic HAUSGARTEN site (ARK XXII-1c, 2007). Cylindrical tubes, laterally covered with a 500 µm mesh, were filled with azoic deep-sea sediment and ^{13}C-labeled food sources (diatoms and/or bacteria). After 10 days of incubation the tubes were analysed for nematode response in terms of colonisation and uptake. Nematodes actively colonized the tubes, however with densities that only accounted for a maximum of 2.13% (51 ind. 10cm^{-2}) of the ambient nematode assemblages. Densities did not differ according to the presence or absence of organic matter, nor according to the type of organic matter added. The fact that the organic matter did not function as an attractant to nematodes was confirmed by the absence of notable ^{13}C assimilation by the colonising nematodes. Overall, colonisation appears to be a process that yields reproducible density and diversity patterns, with certain taxa (e.g. *Sabatieria*, *Thalassomonhystera* and *Leptolaimus*) showing more efficiency. Together with the high variability between the colonising nematode assemblages, this lends experimental support to the existence of a spatio-temporal mosaic that emerges from highly localized, partially stochastic community dynamics.

Chapter 4 aims to understand the processes that shape nematode communities in the deep Southern Ocean, with a special emphasis on characterizing the bentho-pelagic link. Therefore structural and functional aspects of nematodes collected during the ANDEEP-SYSTCO expedition (ANT XXIV-2, 2007/08) were investigated in relation to environmental variables. Samples were collected from six stations along the Prime Meridian (49° S - 70° S)

and included sampling at the abyssal plain, a seamount (Maud Rise) and the continental slope. A repeated sampling was performed at a Polar Front station (52° S) after one and a half months interval to elucidate the short-term response on a seasonal deposit of detritus. Nematode assemblages differed relatively little and were all highly comparable to slope and abyssal communities elsewhere in the world in terms of nematode standing stock, diversity and composition on generic level. The upward migration of nematodes as a response to the recently settled phytodetritus was, however, not reflected in the fatty acid patterns of nematodes. Nonetheless, both dual stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and fatty acids (FA) evidenced coupling between the planktonic food web and deep-sea nematodes at all stations. Gradual shifts in dual stable isotope values and FA compositions of the nematode communities along the Prime Meridian are suggested to relate to variation in the biochemical composition of plankton-derived food sources rather than by changes in community compositions and genus- or species-specific food preferences or metabolic characteristics alone. FA also revealed that bacteria only contribute to a small extent to the diet of deep-sea nematodes and that nematodes may feed year-round on more degraded or constantly available food sources (e.g. fecal pellets, foraminiferans), supplemented by selective feeding on high quality food (phytodetritus) in circumstances of excess fresh labile detritus.

Hydrate Ridge (HR), located on the northeastern Pacific margin off Oregon, is characterized by the presence of outcropping hydrates and active methane seepage. Additionally, permanent low-oxygen conditions overlie the benthic realm. **Chapter 5** explores the relative influence of both seepage and oxygen minima as sources of habitat heterogeneity and potential stress-inducing features on the structure and function of bathyal metazoan benthos (primarily nematodes) at three different seep and non-seep Hydrate Ridge locations. The three locations are exposed to decreasing bottom-water oxygen concentrations with increasing water depth. The nematode seep communities at HR exhibited low diversity with dominance of only one or two genera (*Daptonema* and *Metadesmolaimus*), elevated average individual biomass and $\delta^{13}C$ evidence for strong dependence on chemosynthesis-derived carbon, resembling deep-sea seeps worldwide. Although the HR seep habitats harbored a distinct nematode community like in other known seep communities, they differed from deep-sea seeps in well-oxygenated waters by sharing the dominant genera with the surrounding non-seep sediments overlain by oxygen-deficient bottom water. The homogenizing effect of the oxygen minimum zone on the seep nematode assemblages and surrounding sediments was constant with increasing water depth and concomitant greater oxygen-deficiency, resulting in a loss of habitat heterogeneity.

Chapter 6 situates, integrates and discusses the insights in deep-sea nematode ecology provided by this doctoral study in the context of the current state of knowledge and future work.

In conclusion, this PhD study provides a detailed analysis of nematode structural and functional ecology in different deep-sea ecosystems by means of unique field observations and experiments. The main achievements of this work include that both experimental (^{13}C-tracer) and natural (fatty acids and δ^{13}C, δ^{15}N) observations indicated that bacteria don't play a significant role in the diet of nematodes in photosynthetic driven deep-sea ecosystems. Instead, natural dual stable isotopes and FA evidenced the coupling between the planktonic food web and deep-sea nematodes. Additionally, FA results suggested that nematodes feed year-round on more degraded or constantly available food sources, supplemented with fresh labile detritus in times of excess. At a chemosynthetic driven deep-sea ecosystem, on the contrary, stable isotope results established the link between nematodes and in situ primary production. Furthermore, nematode colonization in the deep sea appears to be a partially stochastic process that yields reproducible abundance and diversity patterns, with certain taxa showing more efficiency. The success of rare genera evidenced that small scale disturbances play a role in establishing and maintaining diversity. It was also shown that a cold seep community that is situated in an oxygen minimum zone is structured differently than cold seep communities in well-oxygenated waters. The oxygen minimum zone seemed to have a homogenizing effect resulting in the occurrence of dominant seep genera in the surrounding non-seep sediments.

Samenvatting

Hoewel het begrip van het leven in de diepzee nog in de kinderschoenen staat, neemt het bewijs toe dat de diepzee een grote diversiteit aan geologische, oceanografische en ecologische settings bevat. Het uiterst heterogene karakter van diepzeehabitats ligt aan de basis van de algemeen hoge diversiteit aan bentische organismen die er gevonden wordt. Mechanismen die leiden tot de coëxistentie van soorten op regionale en lokale schaal zijn echter slecht gekend. Dit verhindert het voorspellen van de toenemende menselijke impact op zowel diversiteit als op het functioneren van diepzee-ecosystemen in het algemeen. Hoewel kleinschalige processen (bv. ruimtelijk-temporele heterogeniteit en verstoring) en kleinschalige, onregelmatige patronen in soortendistributie de basis vormden voor hypothesen over dergelijke mechanismen, bestaat er slechts weinig empirische ondersteuning. Nematoden zijn de meest voorkomende en soortenrijke meercelligen op kleine (cm) en intermediaire (m) lokale ruimtelijke schaal in de diepzeebodem. Samen met hun alomtegenwoordige aanwezigheid, maakt dit ze het taxon bij uitstek om de mechanismen te bestuderen die de verschillende diversiteitspatronen in diepzee-ecosystemen tot stand brengen en behouden.

Door hun numerieke succes en densiteit en biomassa die in het algemeen in verhouding staan tot de beschikbaarheid van voedsel, worden meiofauna, en in het bijzonder nematoden, verondersteld een belangrijke component te zijn in diepzee-voedselwebben. Niettemin blijkt het ontrafelen van hun trofische interacties en functies in diepzeesedimenten niet vanzelfsprekend. Op korte termijn (max. 23 dagen) is de reactie van nematoden op zowel natuurlijke als experimenteel gesimuleerde depositie van phytodetritus slechts minimaal of afwezig in vergelijking met bacteriën, foraminifera en verscheidene macrofauna taxa. Vandaar dat tot op vandaag empirisch bewijs ontbreekt voor de voedingsgewoonten, de voedselvoorkeur, de assimilatiesnelheden en de rol van nematoden in het algemeen, in diepzee-omgevingen die vooral afhankelijk zijn van de toevoer van phytodetritus. Dit gebrek aan kennis belemmert het adequate modelleren van voedselwebben en bentho-pelagisch gekoppelde processen in de diepzee.

Het doel van deze doctoraatsstudie was het verkrijgen van inzicht in het functioneren van benthische diepzee-ecosystemen. Meer specifiek onderzocht deze studie de rol van trofische en oceanografische condities in de ecologie van vrijlevende diepzeenematoden. Experimenten werden uitgevoerd met als doel nieuwe inzichten te verwerven in de rol van bacteriën in het dieet van nematoden en in de respons van nematoden op verstoorde, open stukjes bodem, aangerijkt met organische materie (**Hoofdstuk 2 en 3**). Veldobservaties in zowel fotosynthetisch- als chemosynthetisch-gedreven ecosytemen verdiepten verder in de rol van zowel bacteriën als phytodetritus in het dieet van diepzeenematoden en doelden op het verschaffen van inzicht in de invloed van oceanografische condities op zowel de structuur als de functie van nematodengemeenschappen (**Hoofdstuk 4 en 5**).

Het bredere kader en de belangrijkste kenmerken van dit proefschrift worden geïntroduceerd in **Hoofdstuk 1**.

Hoofdstuk 2 stelt een ^{13}C stabiel isotoop labeling experiment voor dat uitgevoerd werd om het belang van baterieel koolstof as voedselbron voor Arctische diepzeenematoden te quantificeren. In plaats van bacteriën als een 'black box' te behandelen werden verschillende bacterieel, functionele groepen vereikt met verschillende ^{13}C-gemerkte substraten die geïnjecteerd werden in sedimenten verzameld vanop 1280 m diepte op de HAUSGARTEN site (79° N, 6° O, ten westen van Svalbard, ARK XXII-1c, 2007). De opname van het ^{13}C-label in bacteriële, fosfo-lipide afgeleide vetzuren en nematoden vanuit de top 5 cm van het sediment werd gevolgd over 7 dagen tijd. De ^{13}C-dynamiek in nematoden werd gefit met een model om het belang van de verschillende bacterieel functionele groepen als koolstofbron voor nematoden af te leiden. Verschillende patronen in de fosfo-lipide afgeleide vetzuren toonden aan dat de verschillende substraten verschillende bacteriële groepen merkten. De diepzeenematodengemeenschap echter, incorporeerde een zeer beperkte hoeveelheid van het label, waarvan het model duidelijk aangaf dat de dynamiek van de isotopenoverdracht niet te wijten was aan bacterivorie. Bovendien suggereert de lage aanrijking van nematoden op een beperkte passieve opname van de geïnjecteerde substraten. We concludeerden dat opgeloste, organische koolstofbronnen die direct of indirect via bacteriën opgenomen worden van weinig belang zijn als koolstofbron voor diepzeenematoden.

De studie voorgesteld in **Hoofdstuk 3** probeert het belang vast te stellen van infaunale migratie van nematoden in het bepalen van kleinschalige, temporele en ruimtelijke heterogeniteit en de rol van organisch materiaal in dit proces in de diepzee. Daarom werd een kolonisatie-experiment uitgevoerd in situ op 2500 m diepte op de Arctische HAUSGARTEN site (ARK XXII-1c, 2007). Cylindrische buizen, zijdelings bedekt met 500 µm gaas, werden gevuld met azoïsch diepzeesediment en ^{13}C-gemerkte voedselbronnen (diatomeeën en bacteriën). Na 10 dagen incubatie werd het sediment in de buizen geanalyseerd op de nematodenrespons in termen van kolonisatie en opname. Nematoden koloniseerden de buizen actief, maar met densiteiten die slechts 2.13% (51 ind. $10cm^{-2}$) van de achtergrondwaarden bereikten. Densiteiten verschilden niet op basis van de aan- of afwezigheid van organisch materiaal, noch op basis van het type van organisch materiaal dat was toegevoegd. Het feit dat het organisch materiaal de nematoden niet aantrok werd bevestigd door het gebrek aan merkbare assimilatie van ^{13}C door de koloniserende nematoden. In het algemeen blijkt kolonisatie een proces te zijn dat reproduceerbare abundantie- en diversiteitspatronen genereert, waarbij sommige taxa (bv. *Sabatieria*, *Thalassomonhystera*, *Leptolaimus*) efficiënter blijken. Samen met de hoge variabiliteit tussen de koloniserende gemeenschappen, verleent dit experimentele ondersteuning voor

het bestaan van een ruimtelijk-temporeel mosaïek dat ontstaat door zeer lokale, deels stochastische gemeenschapsdynamiek.

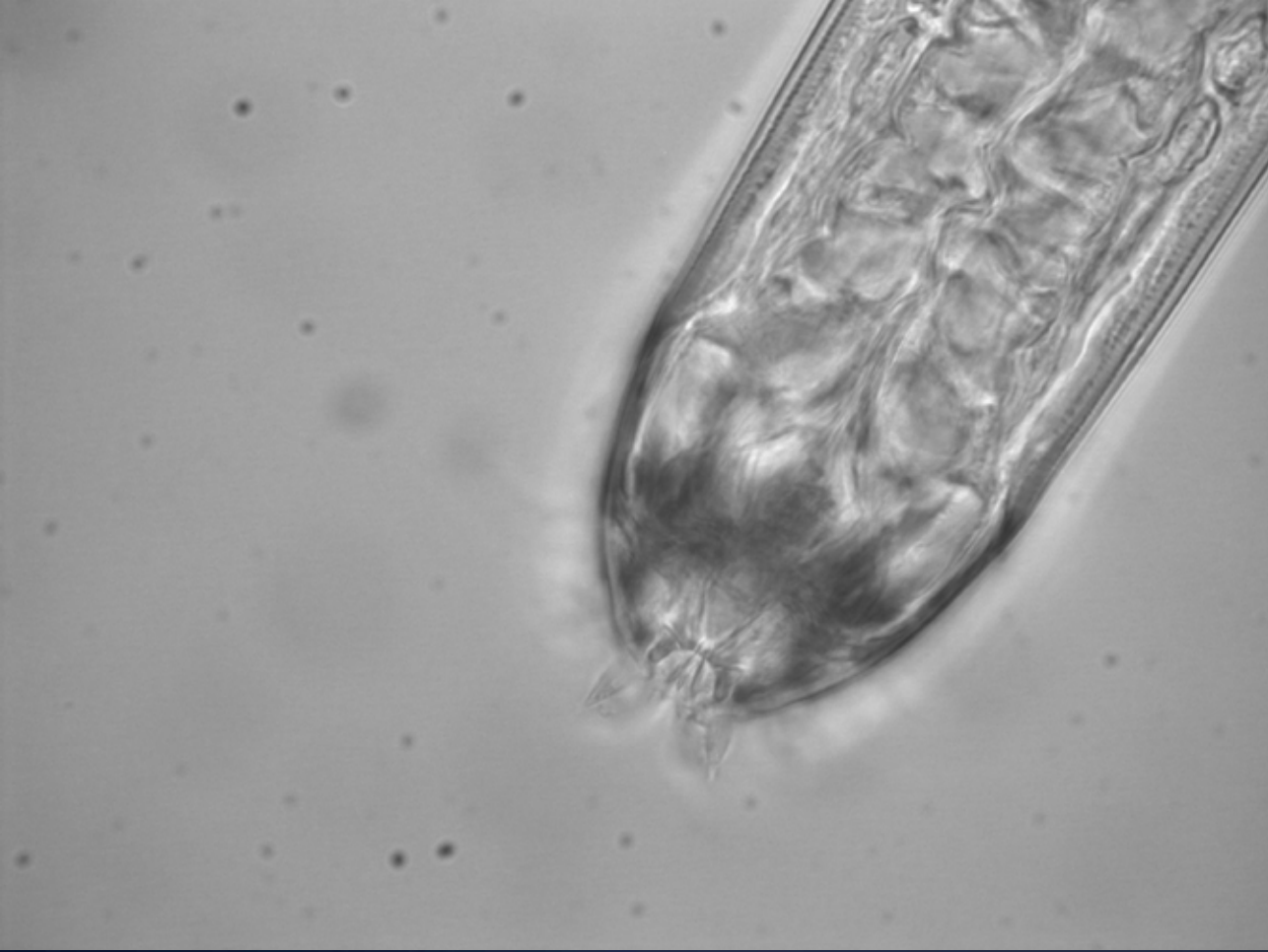
Hoofdstuk 4 streeft ernaar om de processen te begrijpen die nematodengemeenschappen in de diepe Zuidelijke Ocean structuren, met de specifieke nadruk op het karakteriseren van de bentho-pelagische link. Daarom werden structurele en functionele aspecten van nematoden die verzameld werden tijdens de ANDEEP-SYSTCO expeditie (ANT XXIV-2, 2007/08) onderzocht in relatie tot omgevingsvariabelen. Stalen werden verzameld van zes stations langsheen de nulmeridiaan (49° S - 70° S) en bevatten staalnames van abyssale vlaktes, een zeeberg (Maud Rise) en de continentale helling. Een herhaalde bemonstering werd uitgevoerd op een Polair Front station (52° S) na anderhalve maand interval om de korte-termijn respons van nematoden op een seizoenale depositie van detritus na te gaan. De nematodengemeenschappen verschilden relatief weinig en waren allemaal sterk vergelijkbaar met continentale helling en abyssale gemeenschappen elders op de wereld in termen van densiteit, biomassa en compositie op genusniveau. De opwaartse migratie naar het recent neergedaalde phytodetritus reflecteerde zich echter niet in de vetzuurpatronen van de nematoden. Zowel tweevoudige stabiele isotoop ($\delta^{13}C$, $\delta^{15}N$) als vetzuur data bewezen de koppeling tussen het planktonisch voedselweb en diepzeenematoden voor alle stations. Graduele veranderingen in stabiele isotoop- en vetzuurcomposities van de nematoden langsheen het transect suggereerden dat de variatie in de biochemische compositie van planktonafgeleide voedselbronnen aan de basis liggen hiervan, eerder dan veranderingen in gemeenschapscompositie en genus- of soortspecifieke voedselpreferentie of metabolische karakterisitieken alleen. Vetzuren toonden ook aan dat bacteriën slechts weinig bijdragen aan het dieet van diepzeenematoden en dat nematoden zich mogelijks doorheen het jaar voeden met gedegradeerd of constant beschikbare voedselbronnen (bv. fecale pellets, foraminifera), aangevuld met hoog kwalitatief voedsel (phytodetritus) in omstandigheden van overvloed aan vers labiel detritus.

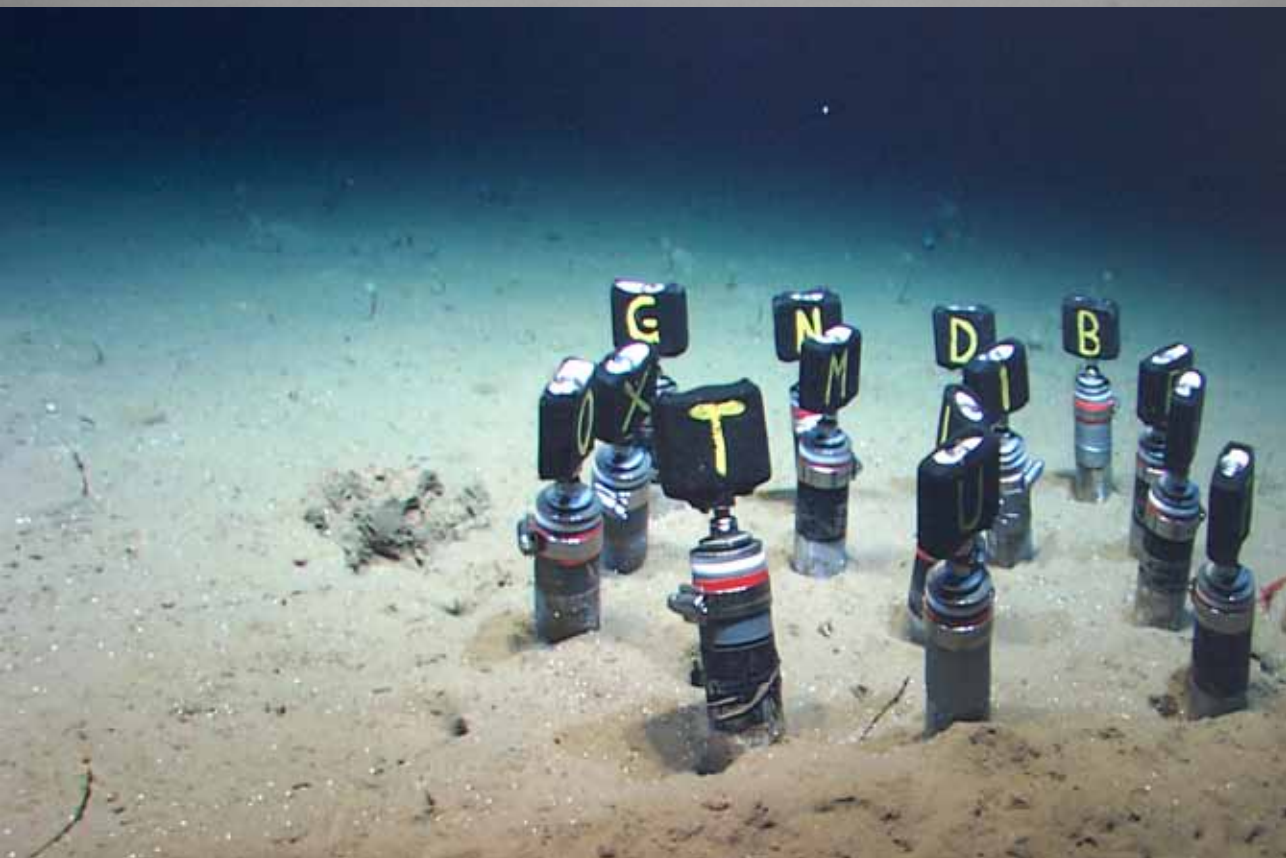
Hydrate Ridge (HR), gelegen op de noordoorst Pacifische continentale rand van Oregon, is gekarakteriseerd door zichtbare aanwezigheid van hydraten en actieve methaanbronnen ('seeps'). Bovendien bedekt water met permanent laag zuurstofgehalte het bentische rijk. **Hoofdstuk 5** onderzoekt de relatieve invloed van zowel seeps als zuurstofminima als oorsprong van habitatheterogeniteit en potentiële stressinducerende factoren op de structuur en functie van het meercellig, diepzeebenthos (hoofdzakelijk nematoden) op drie verschillende seep- en referentielocaties. De drie locaties zijn blootgesteld aan afnemende zuurstofgehaltes in het bodemwater met toenemende waterdiepte. De nematodengemeenschappen van seeps vertonen een lage diversiteit met dominantie van slechts één of twee genera (*Daptonema*, *Metadesmolaimus*), verhoogde gemiddelde individuele biomassa en $\delta^{13}C$-bewijs voor een sterke afhankelijkheid van chemosynthetisch-afgeleid koolstof,

in overeenstemming met diepzee-seeps wereldwijd. Alhoewel de HR seeps een aparte nematodengemeenschap herbergen, net zoals andere seeps, verschillen ze toch van diepzee-seeps in zuurstofrijk water op basis van de gedeelde dominante genera met de omgevende referentiesedimenten die bedekt zijn met zuurstofarm water. Het homogeniserende effect van de zuurstof-minimum-zone op de nematodengemeenschappen van de seeps en omgevende sedimenten was constant met toenemende waterdiepte en gepaardgaand zuurstoftekort, wat resulteert in een verlies van habitatheterogeniteit.

Hoofdstuk 6 situeert, integreert en bespreekt de inzichten in de ecologie van diepzeenematoden voortgebracht uit deze doctoraatsstudie in het kader van de huidige stand van kennis en toekomstige perspectieven.

In conclusie, dit doctoraatsonderzoek geeft een gedetailleerde analyse van de structurele en functionele ecologie van nematoden in verschillende diepzee-ecosystemen door middel van unieke veldwaarnemingen en experimenten. Tot de grootste verwezenlijkingen van dit werk behoort dat zowel experimentele (^{13}C-merker) en natuurlijke (vetzuur en δ^{13}C, δ^{15}N) observaties aantoonden dat bacteriën geen belangrijke rol spelen in het dieet van nematoden in fotosynthetisch gedreven diepzee-ecosystemen. In plaats daarvan bewezen natuurlijke tweevoudige stabiele isotopen en vetzuren de link tussen het planktonische voedselwed en diepzeenematoden. Bovendien suggereerden de vetzuurresultaten dat nematoden zich doorheen het jaar voeden met gedegradeerd of constant beschikbare voedselbronnen, aangevuld met vers labiel detritus in tijden van overvloed. In een chemosynthetisch gedreven diepzee-ecosysteem daarentegen, wezen stabiele isotoopresultaten op een link tussen nematoden en in situ primaire productie. Bovendien, blijkt de kolonisatie door nematoden in de diepzee een deels stochastisch proces te zijn die reproduceerbare densiteits- en diversiteitspatronen creëert, waarbij bepaalde taxa een hogere efficiëntie vertonen. Het succes van zeldzame genera bewees dat kleinschalige verstoringen een rol spelen bij het bepalen en behouden van diversiteit. Er werd ook aangetoond dat een methaanbron-gemeenschap die zich in een zuurstof-minimum-zone bevindt op een andere manier gevormd wordt dan ‘seep’-gemeenschappen in goed geoxigeneerde wateren. De zuurstof-minimum-zone blijkt een homogeniserend effect the hebben die resulteert in het voorkomen van dominante ‘seep’ genera in de omgevende ‘non-seep’ sedimenten.

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General introduction

PROLOGUE

The World Ocean, a continuous body of water divided into several principal oceans and smaller seas, covers 70.8% of the Earth's surface (ca. 3.6×10^8 km²; Pidwirny 2006). Based on the topography of the seafloor, the deep sea is found beyond continental shelves, starting at a depth of about 130 m (Snelgrove 1999). Exceptionally, in Polar Regions where the weight of ice depresses the continents, the shelf break may be more than 600 m below sea level. Overall, deep-sea sediments cover 65% of the world's surface. At the same time it is Earth's least explored and understood ecosystem.

Since the deep sea is an unfriendly environment to humankind, accessing it for research purposes poses considerable challenges. Recent estimates reveal that only 5% of the deep sea has been explored with remote instruments and less than 0.01% of the deep seabed has been sampled and studied in detail (Ramirez-Llodra et al. 2010). In terms of biota, around 91% of the fauna remains undescribed (Mora et al. 2011). These figures illustrate that a rather insignificant proportion of the total deep-sea environment is known and that each new sample carries a high probability of containing a new species.

Although the census of deep-sea life is in its infancy, there is increasing evidence that biodiversity in the deep sea is remarkably high (e.g. Wolff 1977; Grassle and Maciolek 1992; Brandt et al. 2007a, b). At the same time, a wide diversity of geological and ecological settings has been discovered in the past 170 years. Especially in the last 30 years, an increase in discoveries was facilitated by technological developments. Examples are: the improvement of remote sensing using hull-mounted and towed side-scan sonars for high resolution bathymetry mapping and, in particular, the use of submersibles, remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) for direct exploration and experimentation (Ramirez-Llodra et al. 2010).

Today, scientists from a wide range of disciplines are describing abiotic and biotic characteristics of the deep sea at an increasing rate. As a benthic ecologist, my aim is to contribute to the understanding of processes that shape the benthic communities in different trophic and oceanographic settings by studying the relations that living organisms have with each other and with their natural environment. For this doctoral study I focused on the relatively small-sized meiobenthos (sieve mesh limits of 32 - 1000 µm) and in particular on nematodes. Nematodes account for 83% to 89% of total metazoan abundance in deep-sea sediments (Mokievskii et al. 2007) and are one of the most diverse metazoan phyla in the ocean (Lambshead and Shalk 2001; Lambshead and Boucher 2003). From a biological perspective, the deep sea is a dark, cold and high hydrostatic pressure system with generally limited bio-available organic matter (Barber 1968). Therefore, nematodes' high diversity

and increasing relative standing stock with depth (Rex et al. 2006) is modestly expressed 'impressive'. The total sediment surface area sampled for meiobenthos at depths exceeding 1 km was only ca. 10 m² when estimated by Mokievskii et al. (2007). Therefore, it is likely that new data obtained for deep-sea areas may introduce substantial corrections to distribution patterns, or shed new light on ecological theories formulated before on terrestrial, coastal or the relatively little deep-sea data available. Modern sampling and biochemical techniques allowed my research to be performed in ways that was not possible fifteen years ago.

INTERNATIONAL FRAMEWORK

Federal governments should consider increasing investments in research that focuses on the deep sea and more specifically on continental margins because the marine environment is of critical importance to them. The society and economy rely on ocean goods (biomass, bioactive molecules, oil, and gas) and services (climate regulation, nutrient regeneration, and food) that are produced and/or stored along open slopes of continental margins (Danovaro et al. 2009). Additionally, ocean margin ecosystems likely host a large proportion of Earth's undiscovered biodiversity. The exploitation of the deep sea beyond the continental shelf is increasing through bottom-trawl fishing, as well as hydrocarbon exploration and production. However, the deep-ocean environment is still poorly understood. Therefore, several states already came to recognize the need to understand this environment and to assess its vulnerability to anthropogenic inputs in order to regulate it for sustainable exploitation before it is too late. As such, several international projects were designed to provide the scientific knowledge base that will support policy decisions concerning the sustainable management of natural offshore resources and to contribute to the success of a holistic approach to maritime governance (Grehan et al. 2009).

International, European and Flemish funding provided the necessary leverage to several projects that allowed to perform this doctoral study and to work in multidisciplinary teams, both at sea and within partner laboratories. This study was built on productive collaborations that secured the quality of the collected data through the use of infrastructure and equipment that otherwise may not have been accessible.

The European Union's 6th Framework Research Program provided the mechanism to fund the large multidisciplinary project **HERMES** (**H**otspot **E**cosystem **R**esearch on the **M**argins of **E**uropean **S**eas, 2005 - 2009) and its successor **HERMIONE** (**H**otspot **E**cosystem **R**esearch and **M**an's **I**mpact **On E**uropean Seas, 2009 - 2012). On both projects a community of researchers was built that focused not solely on research, but also engaged business partners and developed links to policy makers. The HERMES and HERMIONE consortia were large

enough to enable a fully interdisciplinary approach to European ocean margin research, involving expertise in deep-sea biology, geology, physical oceanography, microbiology, and biogeochemistry. In addition to marine scientists, socio-economists were engaged to bridge the gap between scientists and policy makers, and ecosystem modelers were included to work towards predictive models that could benefit both communities (Weaver and Gunn 2009). HERMES concentrated on "biodiversity hotspots" (cold seeps, cold-water coral locations, carbonate mounds, canyons, and anoxic environments) and included open slopes adjacent to these hotspots in order to compare them to the background. HERMIONE included new areas of research (seamounts and hydrothermal vents) and puts a greater focus on human impacts in the deep-sea environment, with emphasis on the translation of information into policy. HERMIONE also aims for a wider public outreach. The HERMES and HERMIONE objectives addressed in this present doctoral research are related to the open slope ecosystem on the Nordic margin. At the long-term observatory HAUSGARTEN two experiments were performed to contribute to the overall aims of: 1) understanding the relationship between ecosystem and biogeochemical functioning, and biodiversity, 2) forecasting changes in biodiversity and ecosystem functioning linked to global change, by modeling the modern ecosystems and indentifying the key drivers, and 3) understanding the biological capacities and specific adaptations of deep-sea organisms, and investigating the importance of biodiversity in the functioning of deep-water ecosystems.

Through funding of different European countries and the US American Census of Marine Life project, ANDEEP I to III (ANtarctic DEEP-sea benthic diversity: colonisation history and recent community patterns, 2002 - 2005) became international, multidisciplinary pioneering projects in the study of the Antarctic deep-sea benthos. By sampling mega-, macro- and meiofauna of no more than 40 stations in the Atlantic sector of the Southern Ocean, ANDEEP revealed the scale and patterns of unknown species diversity and gained insight in the potential origin of the abyssal Southern Ocean fauna, its degree of endemism as well as species' bathymetric ranges (Brandt and Hilbig 2004; Brandt et al. 2007a, b). **ANDEEP-SYSTCO** (**ANDEEP-SYST**em **CO**upling) started in the austral summer 2007/08 and builds on the precursor programme ANDEEP. Its main goal is to understand the processes that shape the abyssal communities. Therefore SYSTCO aims to investigate functional biodiversity and ecology of abyssal communities of the Atlantic sector of the Southern Ocean, focusing on the role of key organisms, which frequently occur in samples, including their general feeding biology (Brandt and Ebbe 2009). This doctoral research addressed this aim thoroughly with regard to the benthic nematode communities. This was done by means of different biochemical analyses and analyses on structural nematode parameters, covering both spatial and temporal variation.

The Flanders Fund for Scientific Research secures a place on the world map of scientific research through its investments in multidisciplinary, international collaborations such as the

project titled "**Meiofauna at cold seeps: understanding their function, adaptation and origin**" (project number 3G0346, 2007 - 2010). The main aim of this project was to get insight into the biosphere-geosphere coupling processes that regulate and structure the unique cold seep faunal communities in different deep-sea environments. The objectives of this project that were addressed in this doctoral research are: 1) What is driving the biological patchiness at seeps? and 2) What is the trophic position of the meiofauna? The international collaboration with the Scripps Institution of Oceanography (California, USA) provided us access to deep-water cold seep sediment samples from the Hydrate Ridge, which were collected under optimal conditions through ROV and video-guided coring.

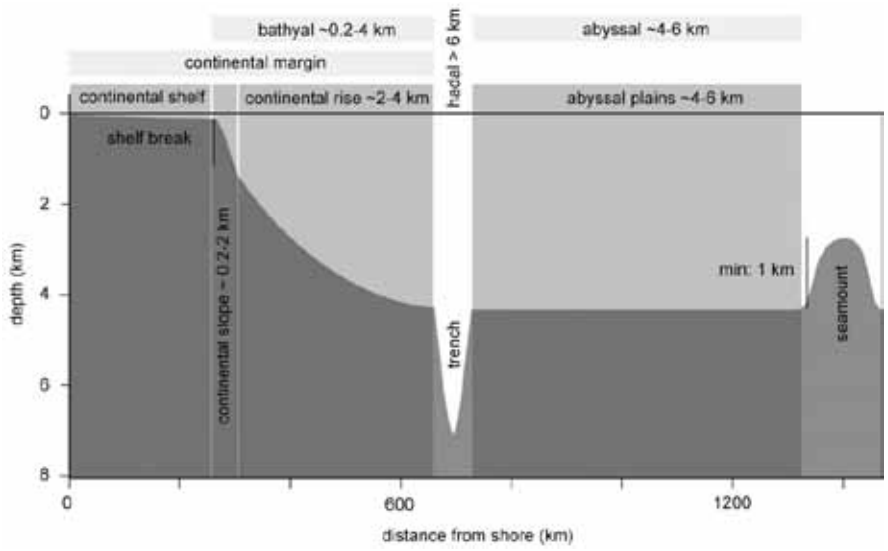
THE DEEP-SEA FLOOR: A MOSAIC OF ECOSYSTEMS AND HABITATS

The ocean, once considered as homogenous and rather food-poor, is now recognized as being highly heterogeneous as a result of tectonic, oceanographic and terrestrial forcing (Levin et al. 2010b). This heterogeneity is manifested as topographic features such as mid-ocean ridges, trenches, seamounts, and continental margins which are interrupted by geological structures like canyons, faults, banks, hydrate ridges, methane seeps, and mud volcanoes. These topographic and geological features further comprise a wide array of ecosystems and habitats that have been discovered at an increasing rate since the start of deep-sea research around 1840 (Ramirez-Llodra et al. 2010). Some examples are whale and wood falls, manganese nodule fields, oxygen minimum zones (OMZ), brine pools, sponge fields, microbial mats, and clam beds. The types of ecosystems and habitats I got acquainted with during this doctoral research are briefly introduced and situated in the following paragraphs.

Abyssal plains account for 76% of the seafloor and are therefore the single largest contiguous feature of our planet. Per definition, these are the regions of ocean crust where slopes and ridge flanks flatten out (ca. 3000 - 6000 m water depth, Fig. 1). Some of the major abiotic characteristics of abyssal plains are relatively uniform: mostly **fine sediments** cover the seafloor (medium sands to clays), temperature varies from -0.5 °C to 3 °C (except in the Mediterranean: 14 °C, and Red Sea: 21 °C); salinity is ca. 35‰ (except in the Mediterranean and Red Sea > 39‰); dissolved oxygen is near saturation: 5 - 6 ml l^{-1} (except in OMZs); pressure increases with 1 atmosphere every 10 m depth; near-bottom flow is constant and low (< 4 cm s^{-1}); and photosynthetically active radiation is absent. Based on these parameters, abyssal plains were long considered to be stable environments where physical and biological processes remained unchanged over short and long time scales. However, evidence accumulated since the 1960s has shown that the expansive ooze bottom is in fact a dynamic

environment (reviewed by Tyler 1988; Gage 2003) experiencing regular (e.g. tidal currents, seasonal deposition of phytodetritus) and episodic (e.g. benthic storms) disturbances~ that may affect the benthic fauna. Although visually often compared with a desert, with only a few obvious detritus feeders (e.g. holothurians and sea urchins) and scavengers (e.g. crustaceans and demersal fish), the top centimeters of sediments of abyssal plains are colonized by very rich communities of macro- and meiofauna (e.g. polychaetes, crustaceans, and nematodes) (Smith et al. 2009).

Figure 1. Profile of typical continental margin and deep-sea system. From McClain and Hardy (2010).



Seamounts are isolated topographic peaks that remain below sea-level but rise more than 1000 m above the surrounding abyssal seafloor, ultimately covering 2.6% of the seafloor (Rogers 1994). In some studies the definition is limited to circular or elliptical features of volcanic origin (Epp and Smoot 1989). Seamounts may occur singly (e.g. Maud Rise in the Weddell Sea), in clusters (e.g. Magellan seamount cluster in the West Pacific), or chains that stretch over considerable parts of the ocean (e.g. the Emperor Seamount Chain in the North Pacific that extends over 6000 km). Their specific topography creates island-like habitats due to their impact on hydrography and subsequent formation of eddies of water (so-called Taylor columns). These eddies are associated with upwelling of nutrient rich deep-ocean waters that lead to increased productivity in upper waters above or downstream of seamounts, influencing both substratum type and diversity of the fauna. Soft sediments are often found at summits of flat-topped seamounts (i.e. guyots) and banks, while the steep slopes generally expose bare rocks (Clark et al. 2010). Other irregular surface features such

as calderas, terraces, pit craters, canyons, caves, pinnacles, knobs, crevices, and cobbles indicate that seamounts also provide a wealth of habitats, with numerous niches, so far poorly studied. While deep-water coral reefs introduce increased complexity to the seamount flanks, soft sediments on terraces or summits host a wide diversity of mostly suspension feeding, epibenthic (e.g. sea pens, sponges, stalked-barnacles, crinoids, isopods) and infaunal (e.g. bivalves, polychaetes, crustaceans, nematodes) organisms. In case of the sediment-dwelling organisms, there is often an inverse relationship between diversity and current strength, because vigorous currents lead to coarser sediments, a poorer habitat and thus smaller diversity of burrowing organisms (Levin and Thomas 1989). Overall, the fauna of seamount habitats is often similar to neighboring areas that fall within organisms' preferred depth ranges (Clark et al. 2010).

The **continental margin** (Fig. 1) is the zone of the ocean floor that stretches from 130 to 4000 m water depth and separates the thin oceanic from the thick continental crust. The transition from the continental to the oceanic crust commonly occurs within the outer part of the margin, called continental rise. The continental shelf is the underwater part of the continental crust which usually abruptly terminates with the continental slope. Overall, active and passive margins account for 10% of the seafloor and are the most geologically diverse areas of the deep-sea floor comprising high habitat heterogeneity (Levin and Dayton 2009; Levin et al. 2010b). Some of the oceanographic, terrestrial and tectonic forcing factors involved in shaping the margin seafloor are: storm-induced waves, currents, internal waves, tidal energy, cascading events, productivity and seasonality of the overlying surface ocean that constrains the supply of nutrients and availability of oxygen in the deep waters, continental sedimentary deposition, landslides, plate subduction and subsequent earthquake and volcanic activity, and compression of buried organic matter. The latter process gives rise to flows of chemically altered fresh- and saltwater and includes biologically or thermogenically induced methane release from cold seeps (Sibuet and Olu 1998; Levin 2005), destabilization of gas hydrates, and large mud volcanoes (Milkov 2000). Each type of margin ecosystem in turn differs based on the coincidence of several of the forcing and abiotic factors. **Cold-seep** ecosystems, for example, appear in a variety of geomorphic and biological forms on the seafloor and as such create a diverse suite of habitats for both endemic seep organisms and more opportunistic colonists (Levin et al. 2003; Cordes et al. 2010). Together with water depth and age of the geologic features, habitat heterogeneity is created by the intensity and volume of fluid flow, as well as methane and sulfide concentrations and fluxes. **Oxygen minimum zones** (**OMZs**), present at intermediate water depths (ca. 100 - 1000 m), also contribute to habitat heterogeneity along continental margins worldwide. This hydrographic feature persists over geological time scales and occurs where upwelling leads to high surface productivity, oxygen-depleted source waters are present, and water stability is

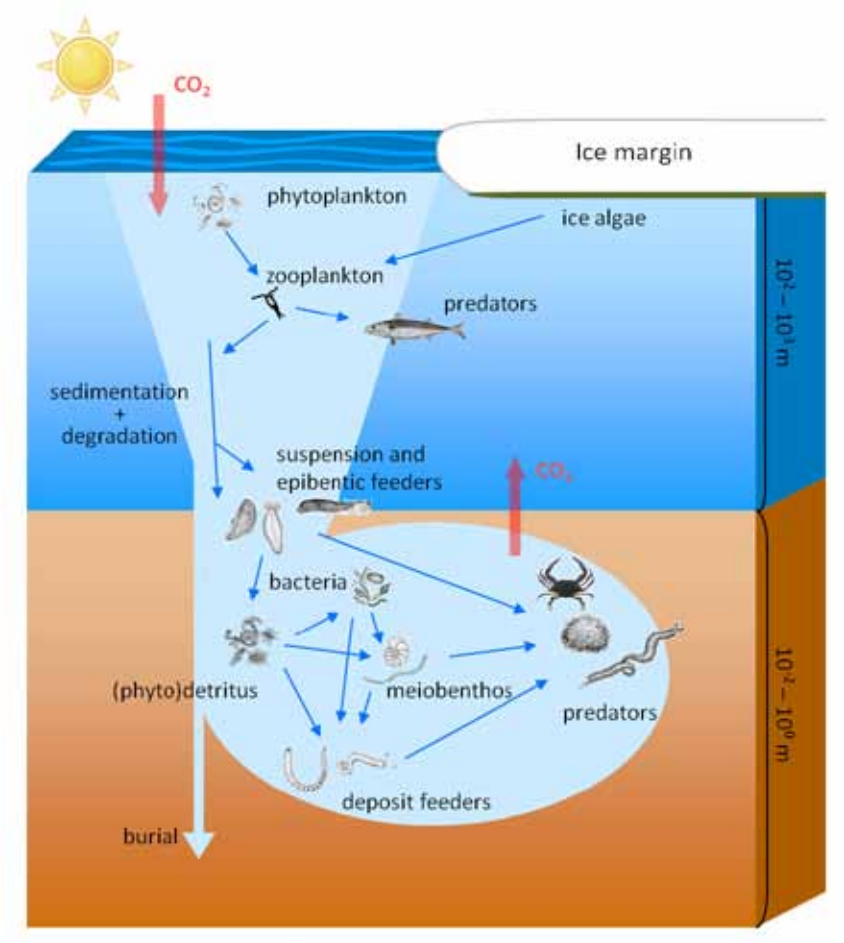
imposed by a strong pycnocline that induces accumulation of settling organic detritus (Levin 2003; Gooday et al. 2010). Typically, O_2 concentrations drop below 0.5 ml l^{-1} or 22 µM O_2. As reduced bottom-water oxygen concentrations enhance preservation of organic matter from the surface (Cowie and Hedges 1992), increased concentrations of labile organic matter and phytodetrital food are found in sediments where the OMZ intersects with the seafloor (often > 3%, up to 20.5% organic carbon; Levin 2003; Gooday et al. 2010). Strong zonation of benthic communities occurs in conjunction with varying bottom- and pore-water oxygen concentrations and associated gradients in organic-matter input that change with water depth or over time (e.g. Wishner et al. 1990, 1995; Levin et al. 1991, 2000, 2001, 2003, 2009; Levin and Gage 1998; Neira et al. 2001). Both at **cold seeps** and **OMZs**, metazoan and protozoan benthic communities are generally distinct from the well-oxygenated surrounding margins and characterized by a reduced diversity (e.g. reviewed in Levin 2003; Levin 2005; Sellanes et al. 2010). At both ecosystems, food limitation is definitely not the constraining factor in structuring the benthos. Other margin areas, however, are generally under influence of a seasonal deposition of detritus that originates from the primary production in the upper water layers. Especially along dynamic **marginal ice zones** (**MIZs**) in Polar regions strong seasonality in light and ice coverage constrains phytoplankton production and subsequent input to margin sediments. In early spring, ice algae grow slowly in a shade-adapted state under the ice. When the ice breaks up and melts, the exponential growth phase begins and the bloom follows the receding ice edge as it melts during spring and summer (Sakshaug and Skjoldal 1989). A peak in sediment-bound pigment concentrations close to the ice edge indicated enhanced primary production in the MIZ (e.g. Bathmann et al. 1991; Schewe and Soltwedel 2003) and was shown to influence the benthic realm in terms of standing stock and diversity in both Arctic and Antarctic margin sediments.

PHOTOSYNTHETIC VERSUS CHEMOSYNTHETIC DRIVEN ECOSYSTEMS

A common characteristic of the deep sea is the lack of photosynthetically active radiation below ca. 200 m (Thistle 2003). Most of the benthic production depends on the input of detrital particulate organic material (POM) produced in the euphotic zone thousands of meters above (Fig. 2). Photosynthetically fixed carbon is transferred to the deep sea through grazing, sinking and active transport by vertically migrating pelagic animals, potentially under influence of lateral advection. Knowledge on the material, quality, quantity and periodicity of the downward flux has been obtained through the use of sediment traps (e.g. Honjo et al. 1980; Lampitt et al. 2008; Bauerfeind et al. 2009) and deep-sea bed photography (e.g. Billet et al. 1983; Lampitt and Burnham 1983; Honjo et al. 1984; Hecker 1990). In some areas of the world

ocean this flux appears as a seasonally predictable pulse (Gage and Tyler 1992). The POM flux manifests mainly in the form of small particles, often aggregated, and including fecal pellets, moults and remains of planktonic animals and phytoplankton and microorganisms. Occasionally, large food falls, consisting of animal carcasses or terrigenous and coastal plant debris occur. Generally, however, the POM flux decreases with water depth and distance offshore (Buesseler et al. 2007) and varies regionally and seasonally with levels of primary production in the upper ocean (Lampitt 1985; Yool et al. 2007). It has been calculated that only 0.5% to 2% of the net primary production in the euphotic zone reaches the deep seafloor below 2000 m (Buesseler et al. 2007). Deep-sea benthic communities are thus among the most food-limited on the globe (Smith et al. 2008), yielding relatively low faunal biomass and productivity (Rex et al. 2006; Rowe et al. 2008). Most studies on the role of food limitation used water-column depth and primary productivity in the overlying water column to postulate that POC fluxes control deep-sea spatio-temporal patterns of faunal standing stocks, and in some cases species richness (Gage 2003; Smith et al. 2008, and references therein). Strong linear relationships between POC fluxes and the abundance and biomass of bacteria, macrofauna and megafauna, however, have been found across regions where the fluxes to the abyssal seafloor were measured directly (Smith et al. 1997; Smith and Demoupolos 2003). Despite the mounting evidence on the presence of seasonal phenomena in the deep sea, studies on benthic responses to the deposition of phytodetritus yielded contrasting results. While bacteria generally react within hours to a few days to the deposition of detritus (Graf 1992; Pfannkuche 1993; Soltwedel 1997), metazoan community changes seemed rather integrated over longer timescales as compared with the seasonality of the fluxes (Gooday et al. 1996; Drazen et al. 1998; Ruhl et al. 2008). This was explained partly due to the differential responses between taxa to environmental and resource variables and competitive interactions (Ruhl et al. 2008).

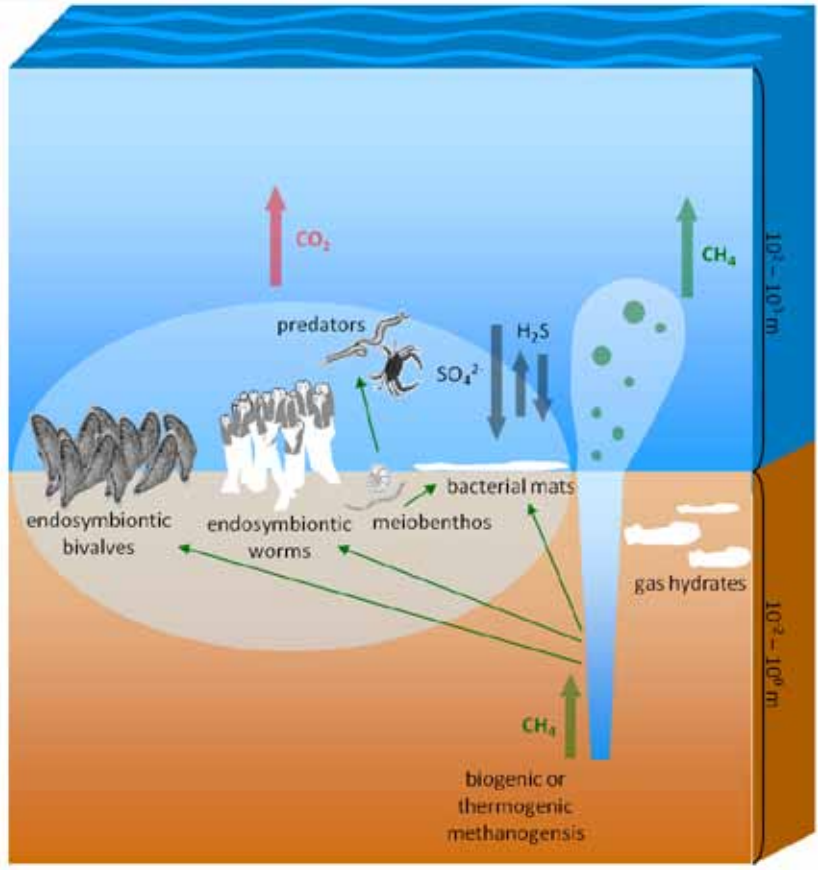
Figure 2. Scheme of a simplified deep-sea benthic food web of a photosynthetically driven ecosystem. Modified from Soetaert and van Oevelen (2009).



As mentioned earlier, the deep sea is not uniformly food-poor. Oases of high productivity occur where organic material from the euphotic zone becomes concentrated by canyons, whale falls, wood falls and OMZs or where seafloor effluxes of chemical energy support intense chemolithoautotrophic primary production (Ramirez-Llodra et al. 2010 and references therein). The latter include hydrothermal vents, pockmarks, brine seeps, mud volcanoes, gas hydrate outcrops and hydrocarbon seeps. In each of these systems, a diverse assortment of aerobic and anaerobic microbial carbon fixation mechanisms, including methane oxidation, ensure a constant and abundant flow of energy to higher trophic levels (Fig. 3). The primary producers that support high biomass of animal communities that are highly distinct from those at the background deep sea, can be both symbiotic and free-living bacteria forming bacterial mats. However, reduced ecosystems are not completely independent of sunlight.

All animals and some microorganisms need dissolved oxygen, which is produced during photosynthesis, for their metabolism. This sets the inevitable link between the surface euphotic layers and all deep-sea communities.

Figure 3. Scheme of a simplified deep-sea benthic food web of a chemosynthetically driven ecosystem. Modified from D. van Oevelen.



Considering the huge coverage of the deep sea and its contribution to the global carbon cycle, it is of great importance to understand the dynamics of organic food consumption and the recycling of this carbon in different deep-sea ecosystems. Moreover, this knowledge will contribute to understand the impact of potential future changes due to climate change. Recent advances in the use of so-called 'inverse modeling' techniques enable modelers to quantify food web flows based on limited data sets and to assess the uncertainty associated with this quantification (van Oevelen et al. 2010). These techniques also allow us to identify what is, or what is not important, and what parts of the system are not being addressed (Gage 2003).

CONCEPT OF BIODIVERSITY

Biological diversity or 'biodiversity' may be considered at several different levels and scales. The basic level is the genetic diversity existing within a species or population. For ecologists the more usual level of biodiversity is the number of species (species richness) or the proportional abundance of each species (evenness). The species count is the simplest of the measures, but only meaningful if it is related to some external measure of sampling size such as number of individuals sampled or area of habitat sampled (Lambshead and Boucher 2003). Neither is it a good measure for analyzing the effect of localized ecological factors on biodiversity because it does not incorporate quantitative information about the relative abundance of each species (Lambshead and Boucher 2003). A wide variety of diversity measures exist that incorporate both richness and evenness but differ in how these components of diversity influence the magnitude of the index (Magurran 1988 in Levin et al. 2001). The choice of an index depends on the nature of the question, the type of data available and the indices used in previous studies with which you wish to compare. The scale at which biodiversity is considered for example (globally or within a more defined area, e.g. regional or local) also plays a role. In most cases, it is useful to use a variety of indices that together provide greater insight into how and why diversity varies than does a single index. Biodiversity may of course also be considered on progressively higher levels of taxonomic hierarchy through genera, families and orders, etc. up to phyla. Examples of the most commonly applied indices in ecological studies on meiofaunal communities are the Hill's indices, that are variably dependant on relative abundances (H_0 = number of genera, H_1, H_2, H_{inf}; Hill 1973) and a rarefaction index, i.e. the expected number of species/genera present in a population of a given number of individuals (ES/G(n); Hurlbert 1971).

At the ecosystem level, biodiversity is addressed in terms of functions of organisms (e.g. production, consumption and transfer of organic matter to higher trophic levels, organic matter decomposition and nutrient regeneration; Danovaro et al. 2008b). This view is more relevant in conservation and habitat management issues, than when biodiversity is solely based on taxonomy (Gage 1996). Moreover, it is the applied approach in studies that focus on e.g. the importance of keystone species in energy flow and the implications of the importance of structure in food webs. The feeding type classification of Wieser (1953) is the most widely used grouping system in studies that deal with nematode feeding ecology. Wieser (1953) classified nematode species/genera in one of four feeding type categories based on their mouth morphologies and related constrains for food types and sizes (1A: selective deposit feeders, 1B: non-selective deposit feeders, 2A: epigrowth or epistratum feeders, 2B: predators/scavengers). Heip et al. (1984, 1985) introduced the trophic diversity index (Θ) to describe nematode communities in terms of the diversity of feeding types. Based

on life strategy, on the other hand, Bongers et al. (1990) allocated marine nematodes on a continuum from colonizers to persisters (r- to K-strategists, sensu lato). The 'cp scale' ranges from one (early colonizers of new resources) to five (persisters in undisturbed habitats). Based on this scale, the maturity index (MI) for a community is the weighted mean cp value of the individuals in a representative sediment sample (Bongers et al. 1990). This index may be applied in studies that describe the successional stages of the recovery of natural communities after disturbance events.

BENTHIC BIODIVERSITY IN THE DEEP SEA

Of the 33 phyla of Metazoa described so far, 31 phyla are found in the sea, 13 of which are exclusively marine (e.g. Echinoderms; Adrianov 2003). Most of the marine Phyla have representatives in marine sediments (Snelgrove 1999). Species diversity in marine ecosystems consists predominantly of invertebrates residing on (epifauna) and in (infauna) sediments (Snelgrove 1999). These invertebrates include large animals (megafauna), such as scallops and crabs, that often can be identified from bottom photographs. However, most species are polychaetes, crustaceans, mollusks (macrofauna, larger than 1000 µm), and nematodes (meiofauna, 32 - 1000 µm). In addition, there are the poorly known microbiota (smaller than 32 µm), which include bacteria, archaea and protists.

What first came as a great surprise in the studies of R. R. Hessler and H. L. Sanders (Hessler and Sanders 1967; Sanders 1968, 1969; Sanders and Hessler 1969), was later repeatedly confirmed by other studies that conducted intensive deep-sea sampling programs (e.g. Wolff 1977; Grassle and Maciolek 1992; Brandt et al. 2007a, b): deep-sea sediments may harbor very diverse faunal communities. Grassle and Maciolek (1992) used the ratio of undescribed to known species to estimate that deep-sea sediments could contain as many as 10 million eukaryotic species, a number that rivals estimates for tropical rain forests (Snelgrove 1999). This estimate generated tremendous debates about the assumptions that were made to scale up species estimates from a relatively small area to ocean basins with estimates of fewer than 500,000 species in total (May 1992), to 100 million nematode species alone (Lambshead 1993) in the deep sea. Most recently, ocean eukaryotic species richness was estimated to be around 2.2 million, of which only ca. 9% has been indexed in a central database (Mora et al. 2011). Regardless of which estimate is correct, more important is to understand the factors that maintain this deep-sea diversity. Considering that the majority of the deep-sea assemblages consist of deposit feeding species that rely on organic detritus for food (Jumars and Eckman 1983), it is perplexing that so many species coexist while exploiting the same limited resource (Levin et al. 2001).

Initially, the 'time-stability' hypothesis of Sanders (1969) invoked long-term equilibrium conditions and competitive niche diversification to explain the relatively high deep-sea diversity (Levin and Dayton 2009). This suggestion was however questioned based on the lack of evidence for niche specialization among species and the parabolic diversity-depth relationship that has been observed for multiple taxa in some areas (e.g. Rex 1981, 1983; Etter and Grassle 1992). With growing evidence of spatial and temporal variability in the deep sea, the focus soon shifted to disequilibrium theories (Dayton and Hessler 1972; Grassle and Sanders 1973; Huston 1979) emphasizing the influence of life-history adaptation, predation pressure, sediment grain size distribution and small-scale physical and biological disturbances on local diversity (Levin and Dayton 2009). Small-scale patches of food, for example, create microhabitats on which different species may specialize and thus avoid competition in a food limited environment (Grassle and Sanders 1973). On the other hand or complementary, it may be the colonization ability and growth rate of individual species, rather than niche complementarity alone that determine the mechanism of coexistence in this type of disturbance. Another example is the disturbance of surface sediments due to the feeding activities of relatively larger organisms. Deposit feeders can have a substantial impact on the surface, both in terms of bioturbation and biochemical reworking (Sibuet and Lawrence 1981; Roberts et al. 2000) and in terms of utilization and redistribution of particulate organic carbon (e.g. Kaufmann and Smith 1997; Miller et al. 2000; Bett et al. 2001). At the same time, some larger benthic organisms may also prey on smaller organisms, potentially altering prey abundances (Iken et al. 2001; Thistle 2003). In general, disturbance of the seafloor is manifested through a variety of biotic and abiotic factors that can affect diversity by regulating levels of competition, predation and physiological stress (Levin et al. 2001). Depending on the severity of these disturbances, the intermediate disturbance hypothesis (Huston 1979) suggests that diversity might be enhanced or depressed. Contemporaneous disequilibrium, in which the deep sea consists of successional patches subject to varied forms of biotic forcing, can further explain how such small-scale, periodic or episodic events that generate heterogeneity in space and time might enhance diversity (Huston 1979). On the other hand, in some areas of the deep sea, diversity is depressed due to overriding environmental variables (Snelgrove 1999). Examples are areas with upwelling-induced hypoxia (Wishner et al. 1990; Levin and Gage 1998; Smith et al. 2000; Levin et al. 2001; reviewed in Levin 2003; Sellanes et al. 2010), hydrothermal fluid emission (reviewed in Van Dover 2000), cold seep methane seepage (reviewed in Levin 2005) and unusually high productivity (Schaff et al. 1992). Overall, when verified with natural occurring diversity patterns, each of the above mentioned hypotheses only partially explains some of the patterns found in the deep sea (Snelgrove and Smith 2002). Moreover, the variability in natural large-scale and experimentally-induced local diversity patterns most likely reflects differences in e.g. the intrinsic heterogeneity of study areas, the available data sets and assumptions used in analyses, and the specific

taxonomic groups examined (Snelgrove 1999). Perhaps most important, it reflects the need to improve sampling coverage and to perform experiments that address the benthic response to disturbance events. When data from a broader range of areas on a global scale and for multiple taxa within the same study area become available through increased sampling efforts, it may be possible to improve on the current depth- and latitude-related generalities and their causes. On the other hand, experiments that address the early arrival and succession of organisms following a disturbance events may provide valuable insight in the establishment, maintance and resilience of diversity in the deep sea. Moreover, it is crucial to study the differences in the underlying coexistence mechanisms since these may lead to differences in biodiversity effects on ecosystem functioning (Loreau et al. 2001).

DEEP-SEA MEIOBENTHOS AND NEMATODES

Benthic animals are usually subdivided into size-based categories. Lower and upper size limits may however vary depending on the size characteristics of the taxon and study area of interest. Deep-sea meiobenthos are generally those animals that are retained by fine screens with mesh openings of 32 µm, and distinguished from the macrobenthos that are retained by sieves with meshes of 1 mm (Rice et al. 1994; Soltwedel 2000). Meiobenthos consists of both multi-celled (metazoan) animals and larger single-celled protozoans, such as Foraminifera. Among the metazoans, Nematoda (roundworms) are probably by far the most numerous in all marine soft bottoms (Gage and Tyler 1992).

Many of the higher meiobenthic taxa were discovered by the mid-nineteenth century (Higgins and Thiel 1988). Wigley and McIntyre (1964) were however, the first to study the meiobenthos beyond the shelf zone (40 to 567 m) off the Atlantic coast of North America. Simultaneously, Thiel started his regular studies of deep-sea meiobenthos on board of the new RV Meteor, off Eastern Africa (Thiel 1966, 1972, 1975, 1979). Since then, data on deep-sea meiobenthos have been gathered from all oceans and ocean depths, which allowed to relate the observed large-scale geographical patterns to various environmental patterns (Soltwedel 2000). Thiel (1983) summarized the quantitative information available for the meiofauna up to 1980. He was the first to demonstrate that the abundance and biomass of meiobenthos decreased with water depth. A decade later Tietjen (1992) presented further data concentrating mainly on information collected during the 1980s. More recent reviews are dedicated to the quantitative distribution of meiobenthos in individual zones of the deep ocean (continental slopes: Soltwedel 2000; abyssal and ultra-abyssal zones: Soltwedel et al. 2003; chemosynthetic environments: Vanreusel et al. 2010a) or integrated over different zones (Vanreusel et al. 2010b). Mokievskii et al. (2007) provided the most recent summary of all data available on the

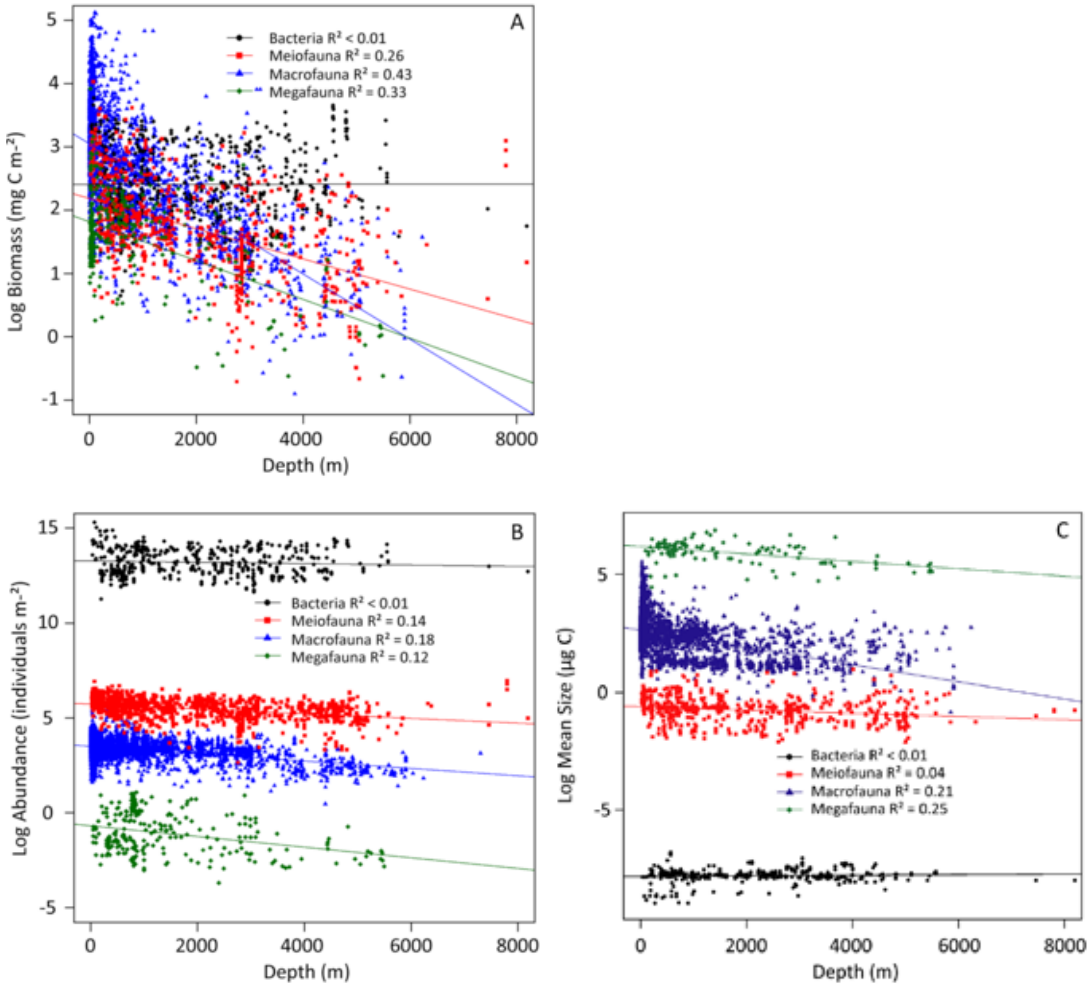
quantitative distribution of meiobenthos from the upper neritic zone to the maximal depths known, to reveal general regularities of its distribution on the global scale. The patterns that emerged from these integrative studies have been tested and refined throughout the years by the supplementation of data. An example is that at higher metazoan taxon level, richer meiobenthic communities tend to develop in shallower areas where there is a higher overall input of organic matter (Soltwedel 2000 and references therein). Additionally, although there is a significant relationship between primary production and metazoan meiobenthic standing stocks (density and biomass), there is large variation in the data, indicating that their relationship is complex. Other factors involved in determining these standing stocks are abiotic parameters (pressure, water temperature, oxygen concentrations, macrotopography, sediment granulometry, pore-water biochemistry), biological processes in the water column (degradation of organic matter in the pelagic food web) and interactions with other faunal groups (competition, predation) (e.g. Soltwedel 2000; Mokievskii et al. 2007; Vanreusel et al. 2010a, b).

Nematodes represent around 85.6% of the meiofaunal relative abundance and are therefore generally the most abundant metazoan taxon in deep-sea sediments (Mokievskii et al. 2007). Moreover, the share of nematodes in metazoan meiobenthos communities increases with the water depth (Mokievskii et al. 2007). Many nematode deep-sea genera are cosmopolitan, inhabiting a variety of deep-sea habitats and oceans, whereas only 21% of all deep-sea genera recorded are restricted to a single habitat (Vanreusel et al. 2010b). Overall, macrohabitat heterogeneity contributes significantly to total deep-sea nematode diversity on a global scale (Vanreusel et al. 2010b).

The role of meiofauna in deep-sea benthic food webs

Patterns of benthic (bacteria, meiofauna, macrofauna, and megafauna) standing stocks are positive functions of surface primary production and input of particulate organic carbon (POC) to the seafloor (Fig. 4A and 4B; Rex et al. 2006; Wei et al. 2010). While bacterial biomass and abundance are highest and remain constant with water depth, meiofauna dominate faunal abundances. In terms of total biomass per surface unit, meiofauna surpass larger size groups such as macrofauna and demersal fish at depths below 3000 m water depth (Rex et al. 2006; Wei et al. 2010). This shift with depth is shown to be affected by the decrease in average body size rather than by abundance, presumably due to decrease in quantity and quality of food supply (Fig. 4C; Wei et al. 2010).

Figure 4. Biomass (A), abundance (B) and average body size (C) as a function of depth for bacteria, meiofauna, macrofauna, and megafauna. The average size was calculated by dividing biomass with abundance. Biomass, abundance and body size were log_{10} transformed and the effects of latitude and longitude were removed by partial regression. From Wei et al. (2010).



In spite of their considerable contribution to the benthic standing stock, an integrative study on cycling of POC along a transect in the Goban Spur area (NE Atlantic, 208 to 4460 m water depth) showed that meiofauna only contribute marginally to total carbon respiration (4%; Heip et al. 2001). In shelf and upper slope sediments more than half of the organic matter flux is respired by macrofauna, while anoxic and suboxic bacterial mineralisation contributes for 21%. The remaining 23% is channeled through nanobiota and oxic bacteria. In lower slope (below 1400 m) and abyssal sediments carbon respiration is dominated by the microbiota

(about 80%), followed by macrofauna (16%), meiofauna (4%) and megafauna (0.5%). Other studies that have partitioned shelf and deep-sea respiration rates among different faunal groups, revealed similar relative proportions. The contribution to total carbon respiration of meiofauna or dominating nematodes varied from < 1% (2500 m, van Oevelen et al. 2011), over 7.5% (8 to 4951 m; Soetaert et al. 2009), and 8% (200 to 1010 m, Piepenburg et al. 1995) to maximum 22% (3700 m, Eldridge and Jackson 1993).

Figuring out what their food sources are is part of the quest to understand the functioning of meiofauna, and in particular nematodes, in deep-sea sediments. This has proved to be an arduous and challenging task. Hitherto, natural observations and isotope-tracer techniques were the generally applied techniques to address this issue. In summary, their short-term reaction to both natural and experimentally simulated depositions of phytodetritus was only minimal or absent compared to bacteria, foraminiferans and several macrofaunal taxa (Gooday 1988; Graf 1992; Pfannkuche 1993; Soltwedel 1997; Levin et al. 1999; Moodley et al. 2000; Galeron et al. 2001; Moodley et al. 2002; Aberle and Witte 2003; Witte et al. 2003a, b; Nomaki et al. 2005; Sweetman and Witte 2008; Ingels et al. 2010a, b). Ingels et al. (2010b) reported a higher uptake of ^{13}C-labeled bacteria compared to ^{13}C-labeled diatoms in two experiments performed in the Arctic and Antarctic region. This uptake was, however, limited to less than 6×10^{-5} mmol C m^{-2} d^{-1}; which is less than 0.1% of the carbon assimilation of 0.06 ± 0.007 mmol C m^{-2} d^{-1} inferred in the study of van Oevelen et al. (2011) for the same Arctic location where Ingels et al. (2010b) performed the experiment. This indicates that although uptake rates of labeled bacteria were higher than labeled diatoms, these labeled carbon sources were insignificant in the total carbon requirements of the nematodes. Therefore to date empirical evidence for deep-sea nematode feeding habits, food preferences, assimilation rates, and in general their significant role, are lacking. This gap of knowledge impedes adequate modeling of deep-sea food webs and bentho-pelagic coupling processes.

Applied molecular techniques to study deep-sea nematode feeding ecology

Stable isotopes (^{13}C/^{12}C, δ^{13}C; ^{15}N/^{14}N, δ^{15}N) are powerful tools in foodweb research. The underlying principle is the enrichment of the heavier isotopes ^{15}N and ^{13}C with each assimilation step in the food chain owing to the selective metabolic loss of the lighter isotopes (^{14}N and ^{12}C) during food assimilation and growth. While carbon isotopic signatures of consumers and resources are typically very similar ($\Delta\delta^{13}$C: 0.5‰), nitrogen isotope ratios show a considerable offset between successive trophic levels ($\Delta\delta^{15}$N: 2.3 - 3.4‰; Van de Zanden and Rasmussen 2001; McCutchan et al. 2003; Post 2002). Hence, dual stable isotope signatures may reveal information on both energy sources and trophic position of consumers,

provided sufficient biomass is available for analysis (Moens et al. 2005a). The requirement of at least 5 µg C or N for most EA-IRMS (elemental analyser - isotope ratio mass spectrometry) analysis, implies the need for around hundred and several hundred nematodes to obtain measurable amounts of C and N, respectively (Moens et al. 2005a). Hence, dual stable isotope ratios of nematodes have hitherto only been reported on community level, with the exception of a few studies on shallow water nematode species (Carman and Fry 2002; Moens et al. 2005a). Stable isotopes have also been used extensively as deliberately added tracers to measure uptake of labeled sources and transfer of energy in marine benthic food-webs. This approach is highly versatile since label can be added as either ^{13}C-DIC or -DOC sources to label the microphytobenthos and/or microbial communities, respectively, in situ, or as ^{13}C-labelled organisms or their remains (such as plant litter or cultured algae) that are cultured under lab conditions before addition (Boschker and Middelburg 2002). Both natural and tracer stable isotope signatures have been implemented in linear mixing models and subsequent linear inverse models to decipher the relative importance of resources (e.g. Levin and Michener 2002; Phillips and Gregg 2003; van Oevelen et al. 2006a, b, 2010; Carlier et al. 2010).

Another method to infer the diet of an organism is based on its fatty acid composition. Fatty acids, in the form of different types of lipids, are essential cellular components of all organisms. They occur as phospholipids in cell membranes and serve, for example, as energy-storage materials in the form of triacylglycerols and wax esters. Certain fatty acids can be used as biomarkers since they are produced in high quantities only by a limited group of organisms and transferred to the consumers without major modification in the consumers metabolism. Examples of biomarkers applied in marine studies are those originating from primary producers such as phytoplankton (e.g. 20:5(n-3), 20:4(n-6) and 22:6(n-3)) and bacteria (e.g. 15:0, 17:0, 15:1, 17:1, *iso* and *anteiso*-branched SFA and MUFA, 18:1ω7, cy17:0 and cy19:0; e.g. Dalsgaard et al. 2003 and references therein; Camacho-Ibar et al. 2003 and references therein). By identifying individual components in a studied organism, fatty acid biomarker analyses allow conclusions to be drawn regarding its food sources, and thus facilitate the direct observation of selective feeding on available organic matter (Suhr et al. 2003). Although this technique is widely spread in marine ecological studies, it was only applied twice on marine free-living nematode species (*Oncholaimus moanae,* Leduc 2009; *Halomonhystera disjuncta,* Van Gaever et al. 2009c).

AIMS AND OUTLINE OF THE THESIS

The overall aim of this study is to gain insight in the functioning of deep-sea benthic ecosystems. More specific, this study addresses the role of food and oceanographic

conditions in the ecology of deep-sea nematodes by means of experiments and field observations at different locations.

The broader framework and key features of this thesis are explained and introduced in **Chapter 1**. Further, the original results of this thesis study are presented and organized in two parts, according to the applied dual approach. The first part comprises **Chapters 2 and 3** which focus on an ex-situ and an in-situ experiment, respectively, performed on a continental slope in the Arctic region. The second part comprises **Chapter 4 and 5** and is based on field observations from the Antarctic Southern Ocean slope and abyssal sediments and a location where an oxygen minimum zone intercepts bare margin sediments and a cold seep area on the northeastern Pacific margin off Oregon, respectively. These two parts are a compilation of research articles that are published or submitted by the candidate as first author. Each chapter is therefore intended to be an autonomous part, which can be read on its own. Inevitably, there may be some overlap between the material and methods of the different chapters. All cited literature is listed at the end of the thesis.

Based on the general minute or lack of response to natural and experimentally simulated phytodetritus fluxes it was speculated that nematodes profit indirectly of detritus by feeding on bacteria. In **Chapter 2** the speculated contribution of bacteria to the diets of deep-sea nematodes is investigated quantitatively by means of an on-board ^{13}C stable isotope labeling experiment. Instead of treating the bacteria as one black box, several bacterial functional groups were isotopically enriched with different ^{13}C-labeled substrates that were injected into sediments collected from 1280 m depth at HAUSGARTEN (79° N, 6° E, west of Svalbard). Incorporation of the ^{13}C label into bacterial phospholipid-derived fatty acids (PLFAs) and nematodes in the top 5 cm of the sediment was monitored over a 7-d period. The ^{13}C dynamics of nematodes was fitted with a simple isotope turnover model to derive the importance of the different bacterial functional groups as carbon sources for the nematodes. This study was published in *Limnology and Oceanography* (Guilini K, van Oevelen D, Soetaert K, Middelburg JJ, Vanreusel A (2010) Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: Results of an isotope tracer experiment. Limnol Oceanogr 55(5): 1977-1989).

The study presented in **Chapter 3** tries to elucidate the importance of nematode infaunal migration in determining small-scale temporal and spatial heterogeneity of nematode community structures and the role of organic matter deposits in this process in the deep sea. Therefore the in-situ ^{13}C stable isotope labeling experiment performed at 2500 m water depth at HAUSGARTEN combined measurements of nematode colonisation and food uptake after 10 days of incubation. This study was published in *PLoS ONE* (Guilini K, Soltwedel T, van Oevelen D, Vanreusel A (2011) Deep-sea nematodes actively colonize sediments, irrespective

of the presence of a pulse of organic matter: results from an in situ experiment. PLoS ONE 6(4): e18912).

Chapter 4 aims to understand the processes that shape nematode communities in the deep Southern Ocean, with a special emphasis on characterizing the bentho-pelagic link. Therefore structural and functional feeding aspects of nematodes collected during the ANDEEP-SYSTCO expedition were investigated in relation to environmental variables. Samples were collected from six stations along the Prime Meridian (49° S - 70° S) and included sampling at the abyssal plain, a seamount (Maud Rise) and the continental slope. A repeated sampling was performed at a Polar Front station (52° S) after one and a half months interval to elucidate the short-term response on a seasonal deposit of detritus. Nematode fatty acids composition and natural stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were determined to provide a time-integrated view on the organisms' diets on community level. This study is submitted in *Progress in Oceanography* (Guilini K, Veit-Köhler G, Mayr C, De Troch M, Van Gansbeke D, Vanreusel A. Evidence for the trophic state and bentho-pelagic coupling of deep-sea nematodes across the Southern Ocean. Prog Oceanogr).

Hydrate Ridge, located on the northeastern Pacific margin off Oregon, is characterized by the presence of outcropping hydrates and active methane seepage. Additionally, permanent low oxygen conditions overlie the benthic realm. **Chapter 5** explores the relative influence of both seepage and oxygen minima as sources of habitat heterogeneity and potential stress-inducing features on the structure and function of bathyal metazoan benthos (primarily nematodes) at three different seep and non-seep Hydrate Ridge locations. The three locations are exposed to decreasing bottom-water oxygen concentrations with increasing water depth. This study is currently in press in *Progress in Oceanography* (Guilini K, Levin LA, Vanreusel A (2011) Cold seep and oxygen minimum zone associated sources of margin heterogeneity affect benthic assemblages, diversity and nutrition at the Cascadian margin (NE Pacific Ocean). Prog Oceanogr, Doi: 10.1016/j.pocean.2011.10.003).

Chapter 6 summarizes and integrates the conclusions of all chapters and suggests future work.

Other published research papers that are of interest to this thesis study and that received a substantial amount of data and writing input from the candidate are provided as supplements in the **Addenda**.

Addendum 1 reports on the response of the deep-sea benthos on the arrival of a fresh phytodetritus flux at an abyssal ANDEEP-SYSTCO station, located in the Southern Ocean Polar Front (2960 m, 52° S 0° E). This study tested the hypotheses that meiofauna and bacteria in oligotrophic Antarctic deep-sea environments react immediately to seasonally deposited

food input with (1) enhanced locomotion and respiratory activity and (2) reproductive effort (increases in number of bacterial cells, production of eggs in copepods, and number of copepod nauplii larvae). My contribution comprises participation in the sampling process, bacterial abundance analysis, and input in writing the introduction and discussion. This study was published in *Deep-Sea Research Part II* as part of a special issue on Southern Ocean biodiversity (Veit-Köhler G, Guilini K, Peeken I, Sachs O, Sauter EJ, Würzberg L (2011) Antarctic deep-sea meiofauna and bacteria react to the deposition of particulate organic matter after a phytoplankton bloom. Deep-Sea Res Pt II 58: 1983-1995).

Addendum 2 explores the differences in the sedimentary characteristics from four stations in the Southern Ocean, along the ANDEEP-SYSTCO transect. This is based on video footage that imaged the sedimentary conditions and epibenthic fauna at the seafloor, and sediment granulometry data for ground truthing. My contribution to this study comprises the sampling and analysis of the sediment granulometry. This study was published in *Deep-Sea Research Part II* as part of a special issue on Southern Ocean biodiversity (Brenke N, Guilini K, Ebbe B (2011) Characterization of the seafloor at the SYSTCO stations based on video observations and ground truthing sedimentology. Deep-Sea Res Pt II 58: 2043-2050).

2

Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: Results of an isotope tracer experiment

Slightly modified from the publication:

Katja Guilini, Dick van Oevelen, Karline Soetaert, Jack J. Middelburg, Ann Vanreusel (2010) Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: Results of an isotope tracer experiment. Limnology and Oceanography 55(5): 1977-1989

ABSTRACT

A ^{13}C stable isotope-labeling experiment was performed in order to quantify the importance of bacterial carbon as food source for an Arctic deep-sea nematode community. The substrates ^{13}C-glucose, ^{13}C-acetate, ^{13}C-bicarbonate and ^{13}C-amino acids were injected into sediments collected from a 1280 m slope station at 79° N 6° E, west of Svalbard, in order to isotopically enrich different bacterial functional groups. Incorporation of the ^{13}C label into bacterial phospholipid-derived fatty acids (PLFAs) and nematodes in the top 5 cm of the sediment was monitored over a 7-day period. The ^{13}C uptake of nematodes was fitted with a simple isotope turnover model to derive the importance of the different bacterial functional groups as carbon source for the nematodes. The different substrates clearly labeled different bacterial groups as evidenced by differential labeling of the PLFAs. The deep-sea nematode community however, only incorporated a very limited amount of the label and the isotope turnover model clearly showed that the dynamics of the isotope transfer could not be attributed to bacterivory. Moreover, the low enrichment of nematodes suggests a limited, and possibly passive, uptake of injected ^{13}C labeled substrates. We conclude that dissolved organic carbon compounds directly or indirectly via bacteria are no important carbon sources for deep-sea nematodes. Since earlier studies with isotopically enriched algae also found limited uptake by nematodes, the food sources of deep-sea nematodes remain an enigma.

Keywords: Nematodes · Bacteria · Deep sea · HAUSGARTEN site · ^{13}C-tracer experiment · In-vitro experiment · Isotope turnover model

INTRODUCTION

Most deep-sea benthic life depends on deposited phytodetritus for energy and carbon requirements (Gooday and Turley 1990; Ruhl et al. 2008; Smith et al. 2008). The detritus dependence of benthic food webs is evident in the relation between water depth and standing stock (biomass and abundance) of metazoan benthos: detritus input decreases with increasing water depth and induces a concomitant decrease in metazoan standing stock (Rex et al. 2006). Bacterial standing stocks in marine sediments are, however, rather constant across water depth (Rex et al. 2006). Their constant standing stock and comparatively high potential production rates imply that they are able to react within hours to a few days to a deposition of detritus on bathyal and abyssal sediments (e.g. Graf 1992; Pfannkuche 1993; Soltwedel 1997). Despite the overall control of detritus input on the benthic food web, it has proven to be more difficult to relate changes in the metazoan standing stock to seasonal inputs of phytodetritus (e.g. Gooday et al. 1996; Drazen et al. 1998; Ruhl et al. 2008) and

to resolve food-web interactions. Metazoan community changes are rather integrated over longer time scales as compared to the seasonal POC deposition, partly because of differential responses between taxa to environmental and resource variables and competitive interactions (Ruhl et al. 2008).

Stable isotope tracer studies, in which isotopically enriched phytodetritus is applied to deep-sea sediments have evidently shown rapid uptake of fresh phytodetritus by the bacterial community within 36 h (Moodley et al. 2002; Witte et al. 2003a) to 8 days (Witte et al. 2003b). Although a comparable direct and considerable ingestion of phytodetritus by protozoan species and several macrofaunal taxa was measured (Levin et al. 1999; Moodley et al. 2000; Aberle and Witte 2003), the short-term uptake by nematodes was limited (Moodley et al. 2002; Ingels et al. 2010a) or absent (Nomaki et al. 2005; Sweetman and Witte 2008; Ingels et al. 2010b). Occasionally a time delay of around three weeks prior to a limited uptake was observed for nematodes (Witte et al. 2003b). As nematodes numerically dominate soft sediment metazoans (Heip et al. 1985) and dominate biomass in deeper sediments (Rex et al. 2006), we are confronted with an interesting enigma: Despite a clear relation between nematode standing stock and water depth (Soetaert et al. 2009), nematodes do not seem to react to inputs of fresh phytodetritus and it is thus unclear what carbon source supports this important component of deep-sea benthic food webs.

A large fraction of the deep-sea nematode genera are, according to their unarmed, small buccal cavity and ecological characteristics, classified as selective deposit feeders or microvores (Moens and Vincx 1997). Jumars et al. (1990) suggested that deep-sea deposit feeders might depend directly on microbes or on their external production because of constraints placed on digesting refractory organic matter in animal guts. Iken et al. (2001) also found $\delta^{15}N$ values of nematodes to be well above $\delta^{15}N$ values of fresh particulate organic matter (POM) at the Porcupine Abyssal Plain. Based on these findings they speculated that bacterivory might be a feeding strategy applied by nematodes. In addition, there are several observations of significant positive correlations between bacterial densities or activity and nematode abundance over a sediment vertical profile and a bathymetric gradient, respectively, in deep-sea sediments (Vanreusel et al. 1995a; Hoste et al. 2007). Although one can speculate that bacteria may contribute significantly to the diets of deep-sea nematodes, there are no quantitative data supporting this potentially important link in deep-sea food webs. The only quantitative data available origin from experiments where freeze-dried, coastal, benthic bacteria were offered to the benthos and only minute uptake by nematodes was observed (Ingels et al. 2010a, b).

Isotope tracer studies in streams (Hall and Meyer 1998; Hamilton et al. 2004) and intertidal sediments (van Oevelen et al. 2006a, b), in which the microbial compartment was isotopically

enriched with a dissolved inorganic or organic substrate, have successfully traced bacterial nitrogen or carbon uptake by bacterivores. A simple isotope turnover model could then be used to quantify the contribution of bacterial carbon or nitrogen in the diet of the consumers (Hamilton et al. 2004; van Oevelen et al. 2006a). In these studies, bacteria were represented as one functional group in the food web, although it is well-known that there are in reality many different active, and inactive, bacterial populations. The most important reason to group bacteria into one functional group has simply been methodological limitations to quantify the transfer of different functional groups of bacteria through the benthic food web. The combined use of isotopically labeled substrates with the quantitative analysis of isotope incorporation into fatty acid (FA) biomarkers made it possible to link bacterial identity to the activity of carbon cycling by different microbial populations (Boschker et al. 1998; Boschker and Middelburg 2002). The basic idea is that an isotopically enriched substrate is administered to the sediment and subsequently incorporated into the biomass and lipid biomarkers of the metabolically active bacterial populations. As the relative amount of lipid biomarkers differs between bacterial populations, the analysis of the full spectrum of lipid biomarkers gives a fingerprint of the active populations. These fingerprints can be compared to infer whether different populations have been active in processing the administered substrate (Boschker and Middelburg 2002).

In this study, we injected ^{13}C labeled substrates into the top 5 cm of deep-sea slope sediments in order to isotopically enrich different bacterial populations and subsequently follow the transfer to nematodes. The substrates were chosen so that they would isotopically enrich bacterial populations that are involved in different biogeochemical processes (Boschker and Middelburg 2002). We added ^{13}C-acetate because it is preferentially oxidized by sulfate reducing bacteria, ^{13}C-bicarbonate since it is a carbon source for chemoautotrophic bacteria such as nitrifiers and sulfide oxidizers, and ^{13}C-glucose and ^{13}C-amino acids because they are more generally taken up by heterotrophic bacteria. The injected ^{13}C label was traced into the bacterial phospholipid-derived fatty acids (PLFAs) which are molecular building blocks of cell membranes. The incorporation into PLFAs was used to evaluate whether indeed the different substrates resulted in discriminate labeling of bacterial populations. In addition, we measured ^{13}C incorporation by nematodes to follow the transfer from bacteria up the food web. The isotope uptake by the nematodes was evaluated by an isotope tracer model to quantify the contribution of bacterial carbon to the nematodes diet. In particular, we focused on the following questions: (1) What is the importance of bacterial carbon in the diet of deep-sea nematodes? (2) Is bacterivory related to depth in the sediment, and therefore to the bacterial abundance or changes in nematode community composition over depth? (3) Does the importance of bacteria as a nematode food source vary with active bacterial populations?

MATERIAL AND METHODS

Study site and experimental approach

The experiment was conducted on board of the German ice-breaker RV Polarstern during the ARK XXII-1c campaign to the HAUSGARTEN site in July 2007. The HAUSGARTEN observatory is located in the Fram Strait (Greenland Sea, west of Svalbard) at the Arctic Marginal Ice Zone and was established by the Alfred Wegener Institute for Polar and Marine Research. The sampling site was located on the continental slope at the eastern, most shallow station (79° N, 6° E) of the long-term, deep-sea observatory site, at 1280 m water depth (HG-I). The samples were collected with a multiple corer (MUC) and immediately transferred to a cold room at in situ temperature (-0.8 °C) and were kept oxygenated by air bubbling. From 3 sequential MUC hauls, 22 cores (8, 7, 7) with an inner diameter of 5.7 cm were recovered. An oxygen penetration depth of approximately 2.5 to 3 cm was measured in two cores immediately after retrieval (C. Rabouille, pers. com.) and the sediment organic carbon content, determined from first centimeter in the experimental control cores, was on average 1.5% or 70 g m^{-2}. After 12 hours of acclimatization the ^{13}C labeled substrates (acetate - "^{13}C-ACE", bicarbonate - "^{13}C-DIC", glucose - "^{13}C-GLU" and an algal amino acid mixture - "^{13}C-AA"; 97% - 99% ^{13}C, Cambridge Isotope Laboratories) were injected in 10 injection wells in the upper 5 cm of the sediment, at regularly spaced intervals, by means of a syringe mounted on an extension rod. The syringe filled with the ^{13}C labeled solution was gradually emptied during retraction from the sediment to achieve a uniform depth distribution of the label. The substrates were injected at a concentration of 60.000 µg C m^{-2} sediment for ^{13}C-ACE, ^{13}C-GLU and ^{13}C-AA and of 180.000 µg C m^{-2} sediment for ^{13}C-DIC (to account for higher background concentrations of DIC as compared to the other substrates). The different substrates were allocated randomly to the cores from the three different MUC hauls. Four control cores without treatment were incubated under similar conditions to investigate background response of the bacterial and meiofaunal communities to the experimental set-up and manipulation of the cores on board. At four time steps (day 1, 2, 4 and 7), one control core and one core of each treatment were taken, sliced per centimeter down to 5 cm and stored until analysis at -20° C. Each centimeter slice of the sediment was sampled for $\delta^{13}C$ of PLFAs, $\delta^{13}C$ of particulate organic carbon (POC), which includes dissolved organic carbon (DOC), and $\delta^{13}C$ of nematodes, and for the nematode community composition. We gave priority to $\delta^{13}C$ samples and therefore had to limit the analysis of the nematode community to the first centimeter slice of the treatment cores, because of low nematode abundance in the deeper slices.

Analytical procedures

Meiofauna was collected from thawed samples via density centrifugation with the colloidal silica polymer LUDOX TM 40 (Heip et al. 1985) and rinsed with tap water. Nematodes that passed through a 1000 µm mesh and were retained on a 32 µm mesh were counted and handpicked randomly with a fine needle. Where possible, 100 nematodes were rinsed in MilliQ water, transferred to a drop of MilliQ water in 2.5 × 6 mm silver cups and acidified with 5% HCl to remove carbonates. Silver cups were pretreated for 6 h at 550 °C in order to remove all exogenous organic carbon. Cups with nematodes were oven-dried at 60 °C, closed and stored in 96-well plates until analysis. An elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) was used to measure stable isotope ratios and carbon content of nematodes. Carbon content was determined from the carbon signal of the EA-IRMS calibrated with Cs_2CO_3 standards. From the upper sediment centimeter from all treatment cores, and from the first five centimeters from the control cores, another maximum of 120 nematodes were handpicked randomly, transferred to anhydrous glycerin and mounted on glass slides for identification to genus level. The community was also analyzed according to the classical division by Wieser (1953) into four trophic feeding guilds: selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A) and predators / scavengers (2B).

Lipids were extracted from 4 g of freeze-dried sediment by means of a Bligh and Dyer extraction, from which the PLFA fraction was isolated. The PLFA extract was derivatized to volatile fatty-acid methyl esters (FAMEs) and measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) for PLFA isotope values (details in Middelburg et al. 2000; van Oevelen et al. 2006a). The bacterial isotope signature was defined as the weighted isotope average[1] of the bacterial PLFA biomarkers (typically saturated and mono-unsaturated PLFAs, ranging from C_{10} to C_{20}; Bergé and Barnathan 2005). PLFAs are present in the membrane and comprise roughly 6% of the total carbon in a bacterial cell. This conversion factor was used to convert PLFA concentration to total bacterial biomass and label incorporation in PLFAs to total bacterial label incorporation (Middelburg et al. 2000). Stable isotope ratios are expressed in the δ notation with Vienna Pee Dee Belemnite (VPDB) as reference standard, and expressed in units of ‰, according to the standard formula: $\delta^{13}C = [R_{sample} / R_{VPDB} - 1] \times 10^3$, where R is the ratio of $^{13}C/^{12}C$ and R_{VPDB} is 0.0111802. Label uptake is reflected as enrichment in ^{13}C and is presented as $\Delta\delta^{13}C$ (‰), which indicates the increase in $\delta^{13}C$ of the sample, as compared to its natural background value, and is calculated as: $\Delta\delta^{13}C$ (‰) = $\delta^{13}C_{sample} - \delta^{13}C_{background}$. Hence, positive $\Delta\delta^{13}C$ values indicate that the organism has acquired some of the introduced

1 Σ (fatty acid specific concentrations [µg C / g sediment] x specific $\Delta\delta^{13}C$ value) / Σ (fatty acid specific concentrations [µg C / g sediment])

label. Absolute uptake of the label (I) is expressed in µg ^{13}C m^{-2} and calculated as: $I = (F_{sample} - F_{background}) \times S$, where F is the ^{13}C fraction: $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R+1)$ and S is the total carbon stock (µg C m^{-2}) of the respective compartment.

Isotope turnover model and parameter calibration

The bacterial carbon contribution in the diet of nematodes was estimated using an isotope turnover model that simulates tracer dynamics in a consumer, given a certain enrichment of its food. The model is given by the following differential equation (Hamilton et al. 2004; van Oevelen et al. 2006a):

$$d\,\Delta\delta^{13}C_{NEM} / dt = k_c \times B_{dep} \times \Delta\delta^{13}C_{BAC} - k_c \times \Delta\delta^{13}C_{NEM}.$$

The first term of the right-hand side of the equation denotes label uptake by the nematodes by grazing on bacteria, and the second term denotes label loss through turnover of the consumer (van Oevelen et al. 2006a). The weighted average isotope values of the PLFAs in the respective depth interval are used as a proxy for the $\Delta\delta^{13}C_{BAC}$ and are imposed as a forcing function. The turnover-rate constant k_c represents the biomass-specific carbon requirements of the consumer, and the bacterial dependence (B_{dep}) determines the fraction of carbon derived from bacteria. B_{dep} ranges from 0, when bacteria do not contribute to the nematode diet and no label uptake by the consumer occurs, to 1, when bacteria fulfill total carbon requirements and the $\Delta\delta^{13}C$ of the nematodes approaches the $\Delta\delta^{13}C_{BAC}$ value with time. The isotope turnover model predicts $\Delta\delta^{13}C_{NEM}$ as a function of the parameters B_{dep} and k_c. Values for both parameters are inferred by changing the model parameters such that the sum of squared deviations between the $\Delta\delta^{13}C_{NEM}$ values predicted by the model and the raw $\Delta\delta^{13}C_{NEM}$ data was minimized. Parameter fitting was done with the R-package FME (Soetaert and Petzoldt 2010) that runs in the R software (R Development Core Team, http://www.R-project.org). The FME package also returns the standard error on the model parameters, which are derived from an estimated parameter co-variance matrix by linearizing around the optimal parameter values. Parameter ranges for k_c were defined based on allometric relations between weight and respiration. Mahaut et al. (1995) related biomass-specific respiration rates (R, d^{-1}) with mean individual dry weight (W, µg C ind^{-1}) for deep-water benthic metazoans:

$$R = 0.0174 \times W^{-0.24}$$

which is valid for the temperature range of 2 - 4 °C. An additional set of weight dependent respiration rates were based on the de Bovée and Labat (1993) formula, corrected for temperature (-0.8 °C) assuming Q10 is 2:

$$R = 0.0449 \times W^{-0.1456} \times \exp^{\ln(Q10)/10(T-20)}$$

where T is temperature (°C). Note that the individual carbon content was directly inferred from the carbon content measured by the EA-IRMS divided by the number of nematodes in a sample cup. The kc parameter range was chosen based on these results (Table 1) and was set to 0.01 - 0.03 d^{-1}.

Table 1. Biomass-specific respiration estimations based on Mahaut et al. (1995) and de Bovée and Labat (1993) with Q10 = 2, used as a proxy for k_c in the isotope turnover model. (Nematode weight is given as an average of replicates ± SD)

		Biomass-specific respiration (d^{-1})	
Depth (cm)	**Nematode weight (µg C)**	**Mahaut et al. (1995)**	**de Bovée and Labat (1993)**
0 - 1	0.035 ± 0.048	0.019	0.018
1 - 2	0.043 ± 0.021	0.016	0.017
2 - 3	0.056 ± 0.039	0.016	0.017
3 - 4	0.073 ± 0.033	0.014	0.016
4 - 5	0.092 ± 0.054	0.014	0.015

Statistics

Statistical analyses were conducted using the PRIMER v6 statistical package (Clarke and Gorley 2006). Analysis on the nematode community composition was based on standardization and Bray-Curtis similarity of square root transformed abundance data of all genera. The Bray-Curtis similarity analyses on the bacterial functional groups were based on log transformed data of the absolute ^{13}C uptake (I) in the bacterial PLFA spectra. Non-metric Analysis of Similarities (ANOSIM), Multi-Dimensional Scaling (MDS) and Principal Component Analysis (PCA) were used to detect and visualize patterns in the nematode and bacterial communities, respectively. The two-way crossed ANOSIM for non-replicated data was performed to test, for example, whether there were among-treatment time differences and treatment differences across all time groups for bacterial absolute uptake (I) data. This double across-group analysis is useful in testing if the differences of the non-replicated time samples for each treatment are responsible for the differences observed between the treatments. A pairwise test in a one-way ANOSIM analysis was then permitted after finding no significant difference ($p > 0.05$) based on the time step grouping, and clarified between what treatments the significant differences were found. Results from the two-way crossed ANOSIM tests are added in Addendum (Appendix A and B).

RESULTS

Nematode community composition and biomass

Nematode densities and biomass in all cores, summed over 5 cm depth and averaged over time, were 1707 ± 112 ind. / 10 cm^2 and 74.4 ± 22 mg C m^{-2}, respectively. A series of two-way crossed ANOSIM analyses showed that in the first centimeter sediment layers of all experimental cores, no differences were found in nematode densities, biomass and community composition between the experimental treatments, across the time steps and vice versa ($R \leq 0.48$, $p \geq 0.018$; Appendix A). Thus, neither the number of nematodes and their biomass, nor the nematode community changed during the experiment. The only differentiation found in the control cores was a significant vertical structuring of the community composition and nematode densities, across the experimental time steps ($R \geq 0.558$, p: 0.001; Appendix B). The pairwise ANOSIM test allocates the vertical community differentiation to significant differences ($R \geq 0.78$, $p < 0.03$) between each sediment depth layer, except for the adjacent ones in the lower three sediment depth intervals where a gradual shift is rather visible (Fig. 1). 44.3% of the total number of nematodes was present in the 0 - 1 cm, 25.9% in the 1 - 2 cm, 21.8% in the 2 - 3 cm, 6.7% in the 3 - 4 cm and 1.7% in the 4 - 5 cm. While densities decreased with depth in the sediment, the mean individual nematode weight (µg C) slightly increased (Table 1), indicating that bigger species inhabited the deeper sediment layers. In the control cores, 77 genera were found, of which the dominant (> 5%) and subdominant (> 1%) genera from the different sediment layers are listed in Table 2. Analysis of the community based on the different feeding guilds showed a gradual increase in relative abundance with depth for the selective deposit feeders (1A) from 35.4% to 56.2% (mainly due to the dominance of *Desmoscolex*), while the abundance of epigrowth feeders (2A) showed a reversed trend and decreased with depth from 35.2% to 8.1% (mainly due to the decreasing presence of *Dichromadora* and *Acantholaimus*) (Fig. 2). The non-selective deposit feeders (1B) and predators and scavengers (2B) showed only small fluctuations in relative abundance with depth, with an average of 30.7% for 1B and 5.4% for 2B.

Figure 1. MDS plot of the nematode community present in the control cores over five centimeters of depth, with indication of the sampling time step (day 0, 2, 4 or 7).

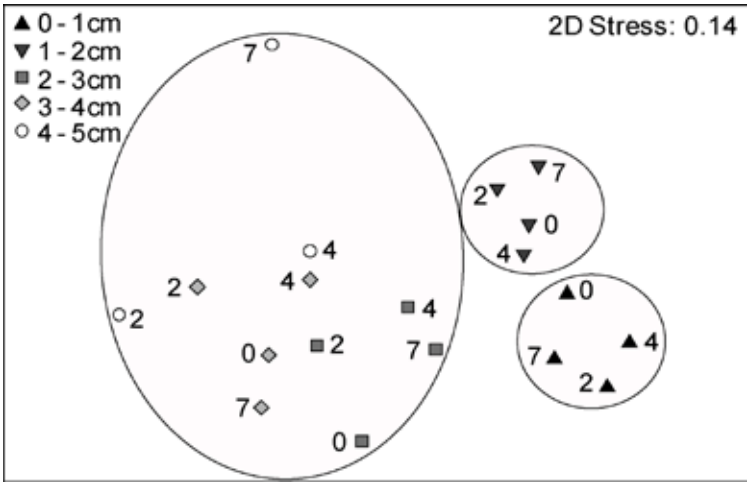


Figure 2. Relative abundance (%) of the feeding guilds according to Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A) and predators/scavengers (2B).

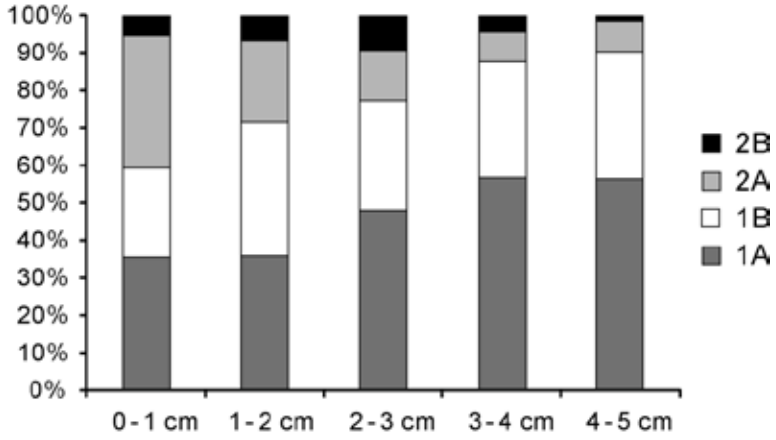


Table 2. Relative abundance of dominant (> 5%) and subdominant (> 1%) genera present in the different sediment centimeter layers from the control cores.

0 - 1 cm	**1 - 2 cm**	**2 - 3 cm**	**3 - 4 cm**	**4 - 5 cm**
Dichromadora 17.7%	*Calomicrolaimus* 10.8%	*Desmoscolex* 20.7%	*Desmoscolex* 32.2%	*Desmoscolex* 24.4%
Desmoscolex 17.2%	*Theristus* 10.1%	*Aegialoalaimus* 8.6%	*Sabatieria* 15.5%	*Sabatieria* 8.9%
Thalassomonhystera 7.7%	*Tricoma* 7.5%	*Monhystrella* 8.1%	*Tricoma* 4.2%	*Theristus* 8.9%
Theristus 7.1%	*Monhystrella* 7.0%	*Sabatieria* 7.3%	*Aegialoalaimus* 4.0%	*Molgolaimus* 6.7%
Calomicrolaimus 6.9%	*Acantholaimus* 6.3%	*Tricoma* 7.1%	*Monhystrella* 3.7%	*Monhystrella* 5.2%
Halalaimus 5.0%	*Amphimonhystrella* 5.5%	*Dichromadora* 6.5%	*Theristus* 3.7%	*Rhabdocoma* 4.4%
Tricoma 4.5%	*Campylaimus* 5.3%	*Sphaerolaimus* 4.5%	*Halalaimus* 3.4%	*Terschellingia* 3.7%
Acantholaimus 4.2%	*Aegialoalaimus* 5.0%	*Theristus* 3.8%	*Sphaerolaimus* 3.4%	*Aegialoalaimus* 3.0%
Campylaimus 4.2%	*Desmoscolex* 5.0%	*Amphimonhystrella* 3.3%	*Calomicrolaimus* 3.1%	*Amphimonhystrella* 3.0%
Anticoma 3.2%	*Halalaimus* 4.3%	*Syringolaimus* 3.3%	*Molgolaimus* 2.8%	*Oxystomina* 3.0%
Chromadora 2.4%	*Dichromadora* 3.3%	*Calomicrolaimus* 2.5%	*Rhabdocoma* 2.8%	*Tricoma* 3.0%
Monhystrella 2.4%	*Sphaerolaimus* 2.8%	*Oxystomina* 2.5%	*Leptolaimus* 2.3%	*Vasostoma* 3.0%
Sphaerolaimus 2.4%	*Daptonema* 2.5%	*Leptolaimus* 2.3%	*Metalinhomoeus* 1.4%	*Campylaimus* 2.2%
Marylynnia 2.1%	*Leptolaimus* 2.5%	*Halalaimus* 1.8%	*Oxystomina* 1.4%	*Leptolaimus* 2.2%
Leptolaimus 1.3%	*Pselionema* 2.5%	*Acantholaimus* 1.5%	*Paralinhomoeus* 1.4%	*Metalinhomoeus* 2.2%
	Retrotheristus 2.0%	*Chromadora* 1.5%	*Wieseria* 1.4%	*Calomicrolaimus* 1.5%
Rest 11.6%	*Thalassomonhystera 1.8%*	*Wieseria* 1.5%	*Amphimonhystrella* 1.1%	*Diplopeltula* 1.5%
	Diplopeltula 1.5%	*Campylaimus* 1.3%		*Halalaimus* 1.5%
	Southerniella 1.3%	*Thalassoalaimus* 1.3%	Rest 12.1%	*Paracantonchus* 1.5%
	Crenopharynx 1.0%	*Axonolaimus* 1.0%		*Pselionema* 1.5%
	Ledovitia 1.0%	*Metadesmolaimus* 1.0%		*Sphaerolaimus* 1.5%
	Oxystomina 1.0%			
		Rest 8.8%		Rest 7.4%
	Rest 10.1%			

^{13}C Incorporation into bacteria and bacterial biomass

Bacterial biomass determined from the concentrations of bacterial PLFAs in all cores, summed over the 5 cm depth interval and averaged over the experimental period, was 738 ± 241 mg C m^{-2}. The two-way ANOSIM showed neither significant difference in biomass between the different treatments, across the time steps, nor between the time steps, across the different treatments ($R \leq 0.091$, $p \geq 0.26$; Appendix A). In other words, the experiment had no influence on the bacterial biomass. Bacterial biomass was highest in the top first sediment horizon with 26% and gradually decreased downwards to 16% in the 4 - 5 cm sediment horizon. Based on the bacterial biomass, the nematode abundances and their biomass specific respiration, it was calculated that 0.03% - 0.22% of the bacterial standing stock would be sufficient to meet nematode respiration requirements. The $\delta^{13}C$ background signatures in the bacterial PLFAs from the control cores ranged from -21‰ to -23‰, without significant differences between the depth intervals.

In each experimental treatment core, ^{13}C labeled substrates were incorporated into bacterial PLFAs within one day after injection and down to 5 cm depth. Total bacterial label incorporation was maximal typically at day 2 and 4 after injection and highest in the top two centimeter (Fig. 3). In the ^{13}C-ACE treatment label incorporation peaked at 3028 µg ^{13}C m^{-2} (5.05% of the added ^{13}C), while in the ^{13}C-GLU and ^{13}C-AA treatment label uptake was comparable and did not exceed 969 µg ^{13}C m^{-2} (1.61%). Label incorporation in the ^{13}C-DIC treatment occurred in only small amounts compared to the other treatments and never exceeded 130 µg ^{13}C m^{-2} (0.07%) and was only observed in the upper two and lowermost cms (Fig. 3).

Figure 4 shows the absolute uptake of the label into the saturated and mono-unsaturated PLFAs. Label uptake peaked at day 2 in most PLFAs in the ^{13}C-AA and ^{13}C-DIC treatments and at day 4 in the ^{13}C-GLU and ^{13}C-ACE treatments. In terms of the amount of label uptake, the same proportions as found for the total bacterial label incorporation were logically reflected here. In terms of PLFA labeling patterns, some PLFA (16:0, 16:1ω7c and 18:1ω7c) were labeled in all, but to a variable extent, while heterotrophic bacteria specific i15:0, ai15:0 and i17:0 were only enriched when ^{13}C was added in organic form (^{13}C-AA, ^{13}C-GLU and ^{13}C-ACE treatments). Based on these treatment related differences in PLFA labeling fingerprints, several bacterial groups could be distinguished. The two-way crossed ANOSIM test indicated a significant difference in PLFA labeling patterns between the treatments (R: 0.91, p: 0.001; Appendix A), across all time steps, while the reverse does not hold true (R: 0.4, p: 0.06). A subsequent pairwise one-way ANOSIM test allocated significant differences between all treatments ($0.93 \leq R \leq 1$). These results are illustrated with a PCA plot (Fig. 5) where the two PC axes explain 93.1% of the observed variation between the different treatments (PC1: 81.4%, PC2: 11.7%).

Figure 3. Total bacterial label incorporation (µg ^{13}C m^{-2}) based on the absolute uptake (I) of the ^{13}C injected substrates over time and depth in the sediment: (A) Glucose, (B) Amino acids, (C) Acetate and (D) Bicarbonate.

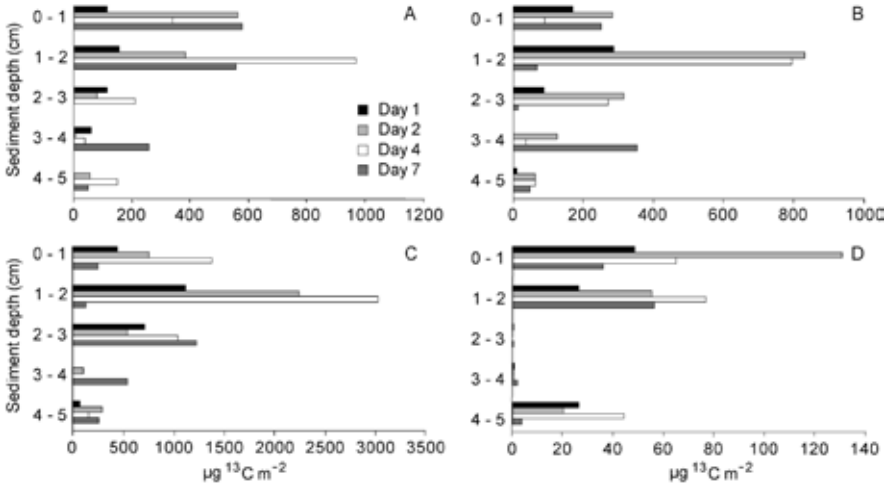
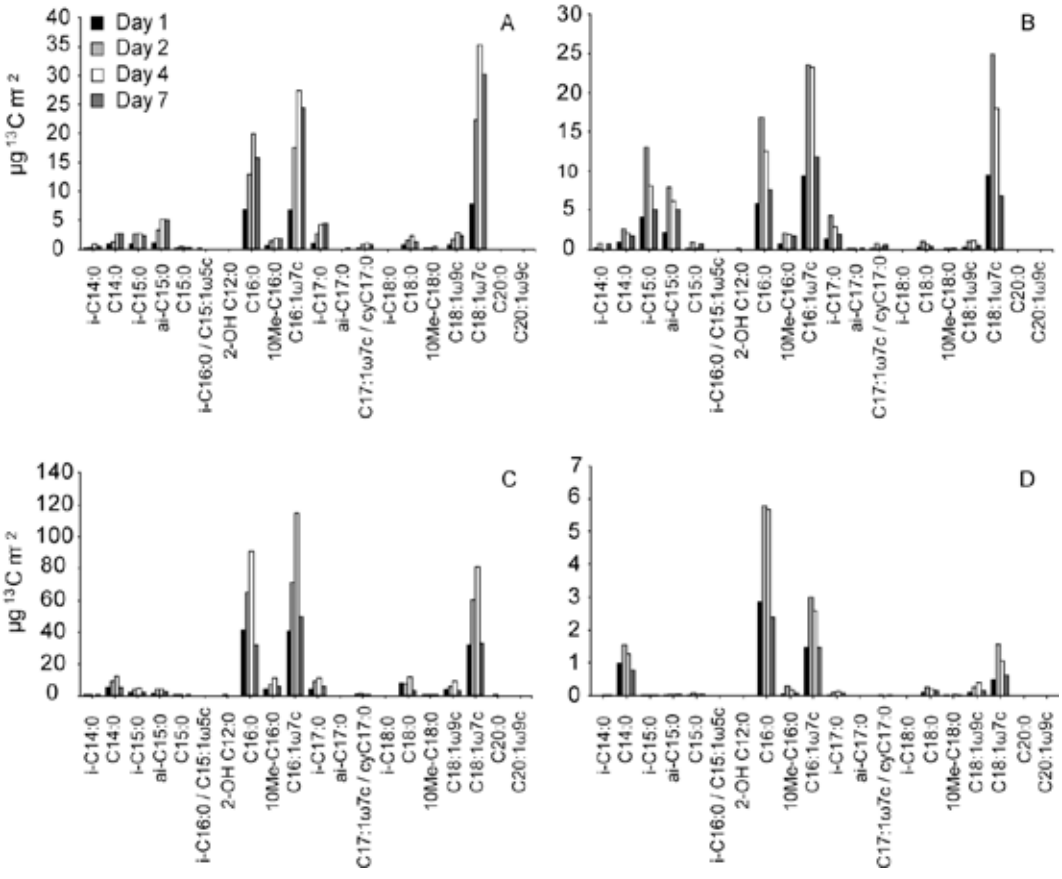
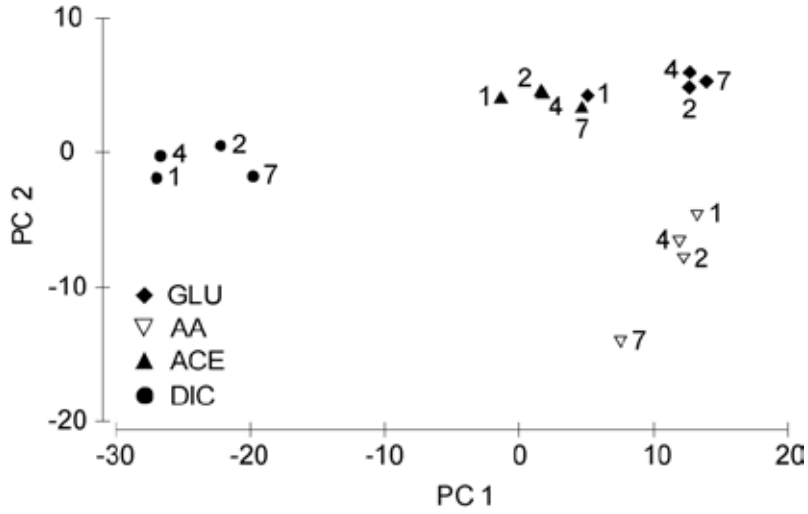


Figure 4. Absolute uptake (µg ^{13}C m^{-2}) of the ^{13}C injected solutions in bacterial PLFA spectra over time: (A) Glucose, (B) Amino acids, (C) Acetate and (D) Bicarbonate.



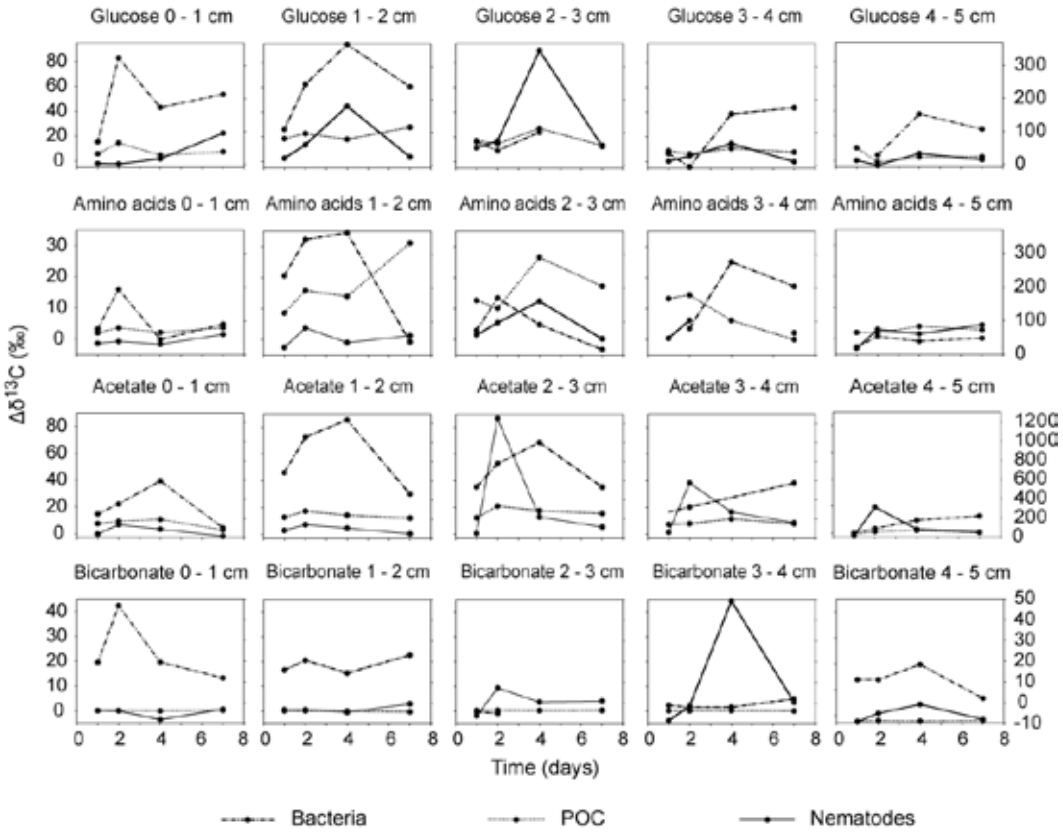
The main contributors explaining the separation of different labeled bacterial groups are 16:0 (-0.765), 18:1ω7c (-0.501), 14:0 (-0.292) for PC1 and 18:1ω7c (0.622), i15:0 (-0.609), ai15:0 (-0.374) for PC2. Chemo-autotrophic bacteria labeled with ^{13}C-DIC were clearly different from heterotrophic bacteria (Fig. 5).

Figure 5. PCA plot of the bacterial PLFA labeling patterns based on the absolute uptake (I) of the ^{13}C label in the different treatments over time, summed over 0 - 5 cm sediment depth, with indication of the sampling time step (day 1, 2, 4 or 7). The two PC axes explain 93.1% of the variation observed (PC1: 81.4%, PC2: 11.7%).



The PLFA ^{13}C isotope values were weighted with their respective concentration to obtain a proxy for bacterial $\Delta\delta^{13}$C dynamics in the different treatments and depth layers, over time. The ^{13}C-DIC treatment distinguished from the other treatments based on the $\Delta\delta^{13}$C values, which did not exceed 46.7‰, while the tracer values in the ^{13}C-ACE treatment peaked at 1238‰ and at 365‰ and 368‰ in the ^{13}C-AA and ^{13}C-GLU treatments, respectively (Fig. 6).

Figure 6. Measurements over time of the $\Delta\delta^{13}C$ (‰) values of bacteria (right axis), and nematodes and POC (left axis), over the different sediment depths, for the different injection treatments: glucose, amino acids, acetate, bicarbonate.

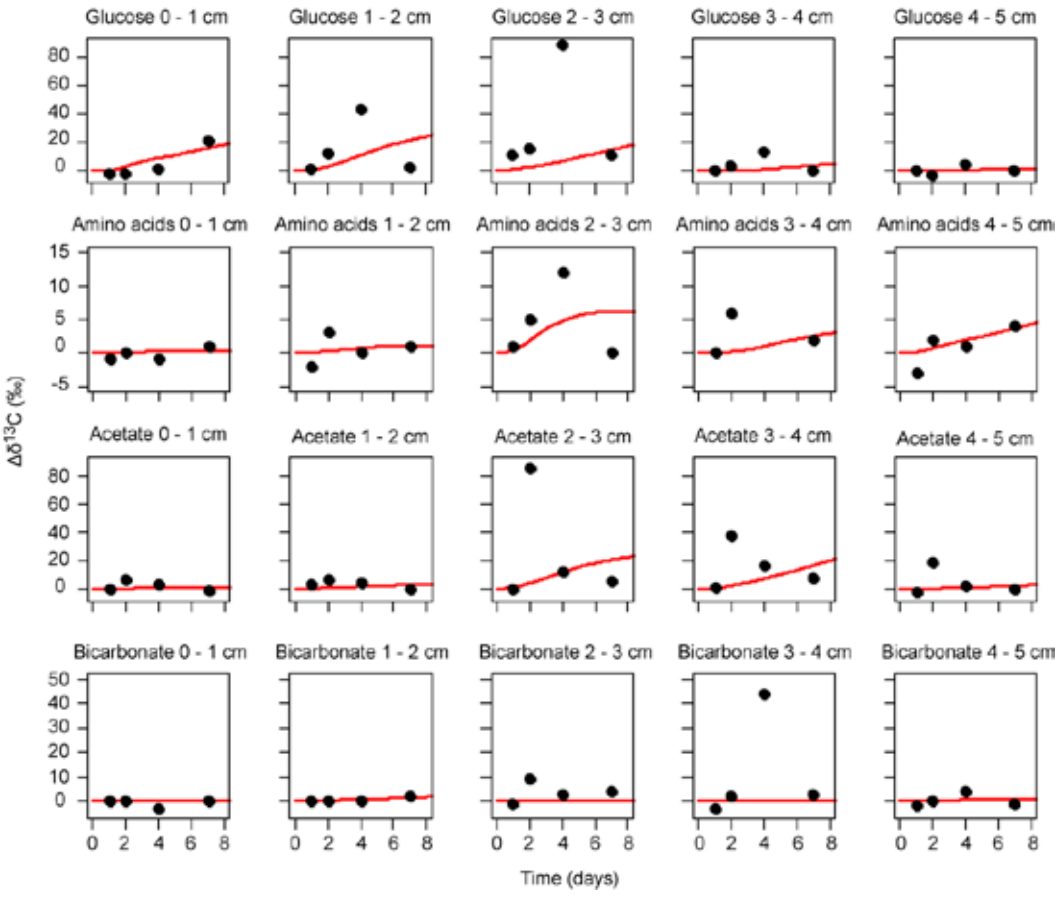


Bacterial carbon contribution to the nematode diet

Natural $\delta^{13}C$ signatures for nematodes ranged from -19.6‰ to -21.9‰ over the 5 cm depth interval and showed a gradual decrease with depth down to 4 cm depth (-19.6 ± 0.4‰ for 0 - 1 cm, -19.9 ± 0.8‰ for 1 - 2 cm, -21.3 ± 2.2‰ for 2 - 3 cm, -21.9 ± 0.8‰ for 3 - 4 cm and -21.3‰ for 4 - 5 cm). The $\Delta\delta^{13}C$ signature of nematodes varied strongly among the treatments and the depth layers (Fig. 6). In some samples, nematodes were labeled well above the background, with a maximum isotopic signature of 89.5‰ in the 2 - 3 cm sediment layer in the ^{13}C-GLU treatment. However, based on the absolute uptake (I) by the nematodes, the number of individuals that were analyzed and their biomass specific respiration, the absolute uptake only accounted for 0.1% - 5.1% of their theoretical daily metabolic requirements (Table 1). Overall, the $\Delta\delta^{13}C$ nematode values were low compared to the bacterial $\Delta\delta^{13}C$ values, suggesting only minor transfer from bacteria to nematodes. In most cases, maximum labeling of nematodes

occurred earlier or coincided with maximum labeling of bacteria, which indicates that isotope transfer to nematodes was not mediated by bacterivory, because a grazing transfer shows as a delayed maximum labeling of the grazer (Hamilton et al. 2004). The theory that the isotope transfer to nematodes is not predominantly mediated by grazing is also substantiated by the model results because it was in most cases not possible to obtain good fits of the data (Fig. 7). Instead, nematode $\Delta\delta^{13}C$ values varied strongly over time and were in the same range as POC $\Delta\delta^{13}C$ values, with which they often co-varied over time (Fig. 6).

Figure 7. Measurements over time of the $\Delta\delta^{13}C$ (‰) values of nematodes and the best model fit, over the different sediment depths, for the different injection treatments: glucose, amino acids, acetate, bicarbonate.



DISCUSSION

Given the ubiquity and high abundance of nematodes in deep-sea sediments, it is essential to identify and quantify the contribution of food sources in the diet of nematodes in order to position them in the deep-sea benthic food web. Some of the earlier hypothesized labile food sources for nematodes in the deep, food-limited seafloor are phytodetritus (as a direct food source), bacterial transformation products of refractory organic matter, bacteria associated with settling phytodetritus, benthic bacteria, fungi and DOC in the pore water (e.g. Rowe et al. 1991; Iken et al. 2001; Smith et al. 2001). Yet, to date there is no evidence that these food sources are of substantial importance to maintain the metabolic demands of these organisms. Food experiments with bacteria, cyanobacteria and diatoms have been performed in different deep-sea locations, but always resulted in a very low uptake by nematodes (Moodley et al. 2002; Witte et al. 2003b; Moens et al. 2007; Ingels et al. 2010a, b). Both the fact that freeze-dried detritus was used and that often a time-lagged response occurred led to the suggestion that nematode uptake could be indirect, through indigenous bacteria or other microheterotrophs growing on the added detritus.

This study tested the hypothesized link between indigenous benthic bacteria and nematodes by means of a pulse-chase experiment using specific ^{13}C labeled solutions in combination with bacterial PLFA screening. The isotope dynamics of the nematodes were evaluated by an isotope tracer model to quantify the contribution of bacterial carbon to the nematodes diet. The experiment was performed on-board with MUC samples being recovered from 1280 m of water depth. It is generally presumed that pressure artifacts due to recovery and handling of the samples at atmospheric pressure are only marginal when samples are recovered from this depth (Jannash and Taylor 1984). Indeed, we observed label uptake measured in PLFAs down to a depth of 5 cm, and the survival of nematodes in equal numbers and biomass, over the course of the experiment. Although substrate injection may not result in a completely homogeneous spread of the substrate in the sediment, it is the preferred method for studying bacterial grazing while maintaining the natural spatial distribution of bacterial and nematode populations (Carman et al. 1989).

Tracer fingerprints of the bacterial community

The fate of the injected ^{13}C labeled substrates was followed over a 7-day period. Label incorporation into the bacterial PLFAs in the upper 3 cm sediment layers peaked at day 2 or 4 and then decreased again. This rapid label uptake indicates an efficient growth on the injected substrate pools in the sediment, while the succeeding decreases in label concentrations towards day 7 suggest high bacterial turn-over rates. The highest incorporation

of the ^{13}C label into bacterial PLFAs took place in the surface 2 cm sediment layers in all experimental treatments. Due to its consistent pattern this can't be attributed to an injection artifact. Instead, it is more likely that the seasonal phytodetritus input associated with the retreat of the ice edge at the HAUSGARTEN location (Schewe and Soltwedel 2003) and the consequential oxygen penetration depth favored the higher bacterial biomass and activity in the upper sediment layers prior to the start of our experiment.

The different ^{13}C incorporation patterns in the PLFAs indicated a successful labeling of different bacterial groups with particular biosynthetic pathways and metabolic specificities (Fig. 5). Active populations can be identified by comparing the biomarkers that are labeled with known biomarker compositions of microorganisms (Boschker and Middelburg 2002). Principal component analysis of the ^{13}C labeling patterns revealed differences between chemo-autotrophic bacteria utilizing dissolved inorganic carbon (bicarbonate) and heterotrophic bacteria using dissolved organic carbon substrates (glucose, amino acids or acetate, Fig. 5). Chemo-autotrophic bacteria using ^{13}C-DIC were strongly ^{13}C-enriched in 14:0, 16:0, 16:1ω7c and 18:1ω7c and incorporated label only in the surface 2 cm with oxygen and at a depth of 4 - 5 cm, but not in between (Fig. 3D). The high ^{13}C enrichment of 16:0, 16:1ω7c and 18:1ω7c suggests that nitrifying bacteria were involved, because nitrifying bacteria from the Schelde estuary showed high incorporation of the ^{13}C label in the PLFAs 16:0, 16:1ω7c and 18:1ω7c (de Bie et al. 2002). The heterotrophic bacteria using amino acids, glucose or acetate were distinct because they also significantly incorporated label in branched PLFAs (i14:0, i15:0, ai15:0 and i17:0) and were also labeled at a depth between 2 and 4 cm. The principal component analysis also revealed a distinct difference between ^{13}C-AA on the one hand and ^{13}C-GLU and ^{13}C-ACE treatments on the other hand, with a more gradual difference between ^{13}C-GLU and ^{13}C-ACE treatments (Fig. 5). Overall, our dataset is too limited for detailed linking of bacterial activities and identities, but it clearly showed that at least four different groups of bacteria were labeled. Hence, nematodes were offered a spectrum of bacterial groups at various depths implying that the observed limited transfer of label to nematodes is likely not due to a lack of adequate bacterial resources.

Nematode community and feeding ecology

The station where the samples were retrieved from for the experiment is part of the long term observatory site HAUSGARTEN and was previously monitored for changes in its nematode community composition over a 5 year study period (2000 - 2004) (Hoste 2006). Only minor differences were found when the time-series was compared with the nematode community from this study. These differences were mainly due to variations in the relative abundance of genera rather than due to the presence/absence of genera. More obvious

than the annual variations were the small-scale changes over the vertical sediment profile, with a gradual increase of selective deposit feeders with depth. Regardless of the vertical differentiation in the sediment, the total nematode community consists of genera with a cosmopolitan distribution in the deep sea, occurring in different habitats and oceans (*Acantholaimus*, *Halalaimus*, *Thalassomonhystera*, *Desmoscolex* and *Theristus*) (Vanreusel et al. 2010b). *Daptonema* and *Sabatieria* are genera contributing to the similarity (> 1%) between several slope areas worldwide (Vanreusel et al. 2010b) and were also found to be dominant or subdominant in some sediment layers of our study area. The community also showed a dominance of nematodes with small unarmed buccal cavities (e.g. *Desmoscolex*, *Tricoma*, *Aegialoalaimus*, *Halalaimus*, *Molgolaimus*), classified by Wieser (1953) as selective deposit feeders, including bacterivores (Fig. 2). These results suggest a maximum presence of 35.4% to 56.2% of bacterivores. Earlier estimates based on mouth morphologies by Jensen (1987a) imply that bacteriophageous nematodes make up 70% (de Bovée and Labat 1993) to 90% (Jensen 1988) in the deep-sea nematode community. In either case, one would expect to see ^{13}C label incorporation in the nematodes of our tracer experiment if these feeding inferences were correct.

In this study, nematode biomass was one order of magnitude lower than bacterial biomass, which was consistent with observations made in several ocean basins (Rex et al. 2006). From bacterial biomass and nematode respiration estimates (Table 1) it is clear that if bacteria are a primary food source for nematodes and the respiration rates are representative, the bacterial standing stocks do not appear to be limiting as the nematodes need approximately 0.03% - 0.22% of the bacterial standing stock to meet their daily respiration requirements[2]. However, from the relatively low nematode $\Delta\delta^{13}C$ values and the lack of a decent model fit we can deduce that the nematode communities in the different sediment depth layers do not depend on bacterial carbon for their metabolism over the observed time span of 7 days. A significant rise in nematode $\Delta\delta^{13}C$ values (corresponding to a high B_{dep}) is often followed by a drop that cannot be explained. If grazing would take place, one would expect accumulation of the label over time. Even in the case where the nematode $\Delta\delta^{13}C$ values reached maximum values, it is unlikely that significant selective feeding on bacteria occurred since the absolute uptake (I) (µg ^{13}C ind^{-1} d^{-1}) by the nematodes only accounted for 0.1% - 5.1% of their theoretical daily metabolic requirements. Our results are in accordance with Baguley et al. (2008) who conducted a 24 h-term in vitro experiment in which aerobic heterotrophic bacteria from the top first centimeter of deep-sea sediments from the Gulf of Mexico were labeled with

2 This is an underestimate of the actual energetic requirements. Based on an assimilation efficiency of ca. 20%, a production efficiency of ca. 80% and assimilation = production + respiration, respiration only accounts for ca. 5% of the actual carbon uptake (based on literature of shallow water, opportunistic, bacterivorous nematodes; Heip et al. 1985).

tritiated thymidine (^{3}HTdr). Scintillation spectrophotometry revealed no bacterial grazing by the nematode community. So neither the classical feeding group classification of Wieser (1953) nor the Jensen (1987a) interpretation, both assuming that a large fraction of the deep-sea nematode community is feeding selectively on bacteria, is experimentally supported. The lack of short-term uptake of the naturally ^{13}C labeled bacterial community also excludes the possibility of bacterivory as an answer to the time lag in detritus uptake observed by Witte et al. (2003a), Moens et al. (2007) and Ingels et al. (2010a) in different pulse-chase experiments. In this regard, the results of this study support other recent ^{13}C stable isotope tracer experiments in deep-sea sediments (Moodley et al. 2002; Ingels et al. 2010b), which all indicate a limited contribution of nematodes to the short-term processing of labeled fresh organic matter. Nevertheless, before reconsidering the expected importance of bacteria and detritus in the diet of deep-sea nematodes, one could still question the respiration rates and response time of nematodes in the deep sea.

The overall slight rise in nematode $\Delta\delta^{13}$C values fell within the range of POC $\Delta\delta^{13}$C values and can therefore be due to uptake of the injected DOC, whether or not passive. In the case of passive uptake, the label could have been measured when just passing the gut, through adsorption or absorption of DOM, by uptake of associated epibiotic bacteria (Ólafsson et al. 1999; and literature therein), or by adsorption of labeled benthic bacteria to the secreted mucus around the body wall of nematodes (Pascal et al. 2008). Another possibility is the deliberate uptake of DOC with immediate use as energy, and release of $^{13}CO_2$ (Levin et al. 1999). If so, this would imply that respiration is an important, unmeasured part of the nematode response in this study. Direct respiration of the simple labile compounds, glucose and acetate, seems feasible since direct uptake by nematodes has experimentally been proven before for both compounds (Chia and Warwick 1969; Montagna 1984; Riemann et al. 1990). However, a similar lack of accumulation of the ^{13}C label was found for the more complex and nitrogen containing amino acids. Since nitrogen is frequently considered a limiting nutrient for benthic organisms (Thomas and Blair 2002 and references therein), one would expect that the labeled amino acids are incorporated and stored when offered in high concentrations in the pore water or accumulated in bacteria. As this is not the case, passive uptake seems the most feasible explanation for the measured label uptake in the nematodes.

Apart from the feeding experiment, the background δ^{13}C signatures of the nematodes give us an indication of the feeding ecology. They change slightly along the vertical profile with more negative values deeper in the sediment. Although these data are probably the result of overlapping signatures of several nematode feeding guilds and nematode genera, they could indicate a depth-related difference in feeding strategy in the sediment, with more dependence on specific fresh, high quality components in the upper centimeter layers and more refractory organic matter deeper down the sediment profile where more degraded organic matter is

present. Future stable isotope feeding experiments under controlled conditions and biomarker studies on alternative potential food sources (e.g. flagellates, ciliates and nannobiotic fungi), preferably on lower nematode taxonomic levels, are recommended before an ecologically valid grouping into feeding guilds and a proper assessment of their position in the benthic food web can be made.

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ADDENDUM

Appendix A. Results from the two-way crossed ANOSIM tests for differences in nematode densities, biomass and community composition between the first centimeter of all experimental treatments and for differences in bacterial biomass and PLFA fingerprints between the summed five centimeter of all experimental treatments. Significant results are indicated in bold.

Factor	Global test	Nematode Density	Nematode Biomass	Nematode community composition	Bacterial biomass	Bacterial PLFA fingerprints
Treatment	R	0.325	0.006	- 0.119	0.073	**0.914**
	p	0.038	0.402	0.756	0.282	**0.001**
Time step	R	0.480	0.206	- 0.149	0.091	0.400
	p	0.018	0.102	0.770	0.257	0.055

Appendix B. Results from the two-way crossed ANOSIM tests for differences in nematode densities, biomass and community composition over the 5 cm depth layers between the control samples. Significant results are indicated in bold.

Factor	Global test	Nematode Density	Nematode Biomass	Nematode community composition
Depth layer	R	**0.937**	0.011	**0.558**
	p	**0.001**	0.391	**0.003**
Time step	R	0.114	0.091	0.491
	p	0.148	0.231	0.014

3

Deep-sea nematodes actively colonize sediments, irrespective of the presence of a pulse of organic matter: results from an in-situ experiment

Slightly modified from the publication:

Katja Guilini, Thomas Soltwedel, Dick van Oevelen, Ann Vanreusel (2011) Deep-sea nematodes actively colonize sediments, irrespective of the presence of a pulse of organic matter: results from an in situ experiment. PLoS ONE 6(4): e18912

ABSTRACT

A colonization experiment was performed in situ at 2500 m water depth at the Arctic deep-sea long-term observatory HAUSGARTEN to determine the response of deep-sea nematodes to disturbed, newly available sediment patches, enriched with organic matter. Cylindrical tubes, laterally covered with a 500 µm mesh, were filled with azoic deep-sea sediment and ^{13}C-labeled food sources (diatoms and/or bacteria). The tubes were inserted in the sea floor by ROV and retrieved after 10 days. The sediment in the tubes was analysed for nematode response in terms of colonization and food uptake. Nematodes actively colonized the tubes, however with densities that only accounted for a maximum of 2.13% (51 ind. $10cm^{-2}$) of the ambient nematode assemblages. Densities did not differ according to the presence or absence of organic matter, nor according to the type of organic matter added. The fact that the organic matter did not function as an attractant to nematodes was confirmed by the absence of notable ^{13}C assimilation by the colonizing nematodes. Overall, colonization appears to be a process that yields reproducible abundance and diversity patterns, with certain taxa showing more efficiency. Together with the high variability between the colonizing nematode assemblages, this lends experimental support to the existence of a spatio-temporal mosaic that emerges from highly localised, partially stochastic community dynamics.

Keywords: Nematodes · Deep sea · ^{13}C-tracer experiment · In-situ experiment · Disturbance · Colonization · Patch dynamics

INTRODUCTION

Vast deep-sea soft-sediment areas that appear to be static and monotonous are in fact subject to a variety of natural disturbances. The spatial extent of disturbance and the temporal scales at which disturbed patches are produced range from small-scale, often ephemeral tracks, pits, tubes and faecal mounds, through intermediate-scale seasonal phytodetritus falls, benthic storms and dense water cascading events, up to large-scale events like turbidity storms or land slides (Thistle 1981; Levin et al. 2001). These disturbances create a spatial and temporal mosaic in which modified conditions and often newly available habitats provide the opportunity for colonization (Johnson 1970). According to the patch-mosaic model, each of these patches evolves separately through a succession process driven by varied forms of biotic forcing (colonization ability, reproductive success, growth efficiency, biological interactions, niche diversification, larval dispersal capacity, and other life strategies), and as such enhances the coexistence of a large number of species (Grassle and Sanders 1973). To verify the model and to understand the processes that structure the highly diverse benthic

deep-sea community, basic knowledge on rates and patterns of faunal colonization and succession is crucial (Gage 1991).

Perhaps the most important factor structuring the soft-sediment community is the spatio-temporal input of organic matter, originating from the phototrophic surface of the oceans (Gooday 2002a; Ruhl et al. 2008). The input can be considered as a disturbance due to its seasonal and patchy character, but at the same time as the energy source that sustains the benthic community (Smith 1986; Grassle and Morse-Porteous 1987). Bacteria, Protozoa and several macrobenthic taxa have shown a rapid natural response (Soltwedel 1997) and considerable uptake of phytodetritus in ^{13}C-tracer experiments that simulated pulses of phytodetritus (Levin et al. 1999; Moodley et al. 2002). Instead, the reaction of the deep-sea metazoan nematodes is relatively slow (Ruhl et al. 2008) and uptake of ^{13}C-labeled phytodetritus was always limited (Moodley et al. 2002; Ingels et al. 2010a) or absent (Nomaki et al. 2005; Sweetman and Witte 2008; Ingels et al. 2010b). This implies that nematodes that dominate abundance, biomass and probably local species richness amongst the metazoan meiofauna (Heip et al. 1985; Lambshead 1993), display a trophic specialisation and/or slow biological processes that avoid competitive displacement (Giere 2009). To understand the success of deep-sea nematode communities, it is necessary to first investigate their ecology and the principles of succession processes, starting with the early arrival of species in newly available patches.

Colonization experiments have been used widely to study the impact of disturbance, and in particular food deposition, on benthic community structures (Zhou 2001, and references therein). Mobile macrofauna in both deep and shallow areas are rapidly attracted to experimentally deposited food. As such, macrofaunal colonization of food-enriched patches generally results in initially higher abundances and lower diversity compared to the ambient sediment (e.g. Grassle and Morse-Porteous 1987; Snelgrove et al. 1992; Quijón et al. 2008; Menot et al. 2009). Nematodes may also recolonize and reach background densities within hours or days after disturbance (Sherman and Coull 1980; Billheimer and Coull 1988; Savidge and Taghon 1988). However, this has only been demonstrated in shallow and intertidal sediments where wave turbulence and tidal currents are strong enough to enable resuspension and passive distribution of nematodes through the water column. Nematode colonization in deep and low energy environments is not stimulated by physical transport, but instead relies on active migration by nematodes. Nematodes with a large body size can more easily burrow through the sediment (Tita et al. 1999) and might therefore have greater colonization abilities via infaunal migration compared to smaller congeners (Schratzberger et al. 2004; Gallucci et al. 2008b). Gallucci et al. (2008b) performed an on-board microcosm study to investigate active colonization of defaunated Arctic sediments from 1300 m water depth. The defaunated sediments remained unenriched or were enriched with the diatom

Thalassiosira weissflogii (1 g organic C m^{-2}). Nematodes colonized both sediments with mean abundances corresponding to 5% (unenriched) and 20% (enriched) of mean nematode abundances in the controls after 9 days. Nematode abundance was significantly higher in the enriched sediments, suggesting fresh detritus enhances colonization and/or resilience. Colonization rates measured in this experiment were similar to, or at the lower end of reports on small-scale infaunal migration into azoic sediments by nematodes in subtidal areas at comparable time scales (Alongi et al. 1983; Chandler and Fleeger 1983; Sherman et al. 1983; Widbom 1983; Ólafsson and Moore 1990). However, the majority of the colonizing species were rare or undetected members of the background sediments and the colonizing community differed strongly between the defaunated sediments. The significantly higher colonization of the enriched sediments seems to be in contrast with the results from the ^{13}C-phytodetritus experiments, where uptake rates of deep-sea nematodes are consistently low (see above). However, given that mostly rare species responded with rapid colonization in the experiment by Gallucci et al. (2008b), their uptake in the ^{13}C-phytodetritus experiments may have been masked by the majority of less responsive nematodes.

To elucidate the importance of nematode infaunal migration in determining small-scale temporal and spatial heterogeneity and the role of organic matter deposits in this process in the deep sea, this study combined measurements of nematode colonization and food uptake. More specific, we compare the short-term, in-situ response of deep-sea nematodes to different pulses of organic matter in terms of colonization and food uptake. The response was monitored by inserting experimental tubes into the sediment at 2475 m water depth by means of ROV manipulation. The tubes were laterally covered with a 500 µm mesh and contained azoic sediments. In addition to undisturbed sediment, four ^{13}C-labeled potential food sources were used: 1) the diatom *Thalassiosira pseudonana*, 2) the diatom *Skeletonema costatum*, 3) benthic bacteria and 4) bacteria grown on degrading diatoms (only bacteria were labeled, see below). Diatoms were selected to determine colonization of patches with settled labile phytodetritus, associated with medium scale disturbance. Bacteria are attached and involved in the decomposition of settling particulate organic matter, and were therefore also considered as a potentially favoured food source. Benthic bacteria were chosen as they represent a more stable background resource, with constant biomass and abundance in marine sediments (Rex et al. 2006). The following hypotheses were tested:

(H_1) Deep-sea nematodes colonize disturbed, newly available habitats.

(H_2) The presence of fresh food enhances the rate of colonization and is reflected in higher nematode abundances and numbers of coexisting genera.

(H_3) Mainly rare nematode species colonize the experimental, enriched tubes and food consumption is evident in strong isotope enrichment as compared to earlier ^{13}C-phytodetritus

experiments that monitored the response of the bulk community.

(H_4) The colonizing nematodes show preference for an added type of food.

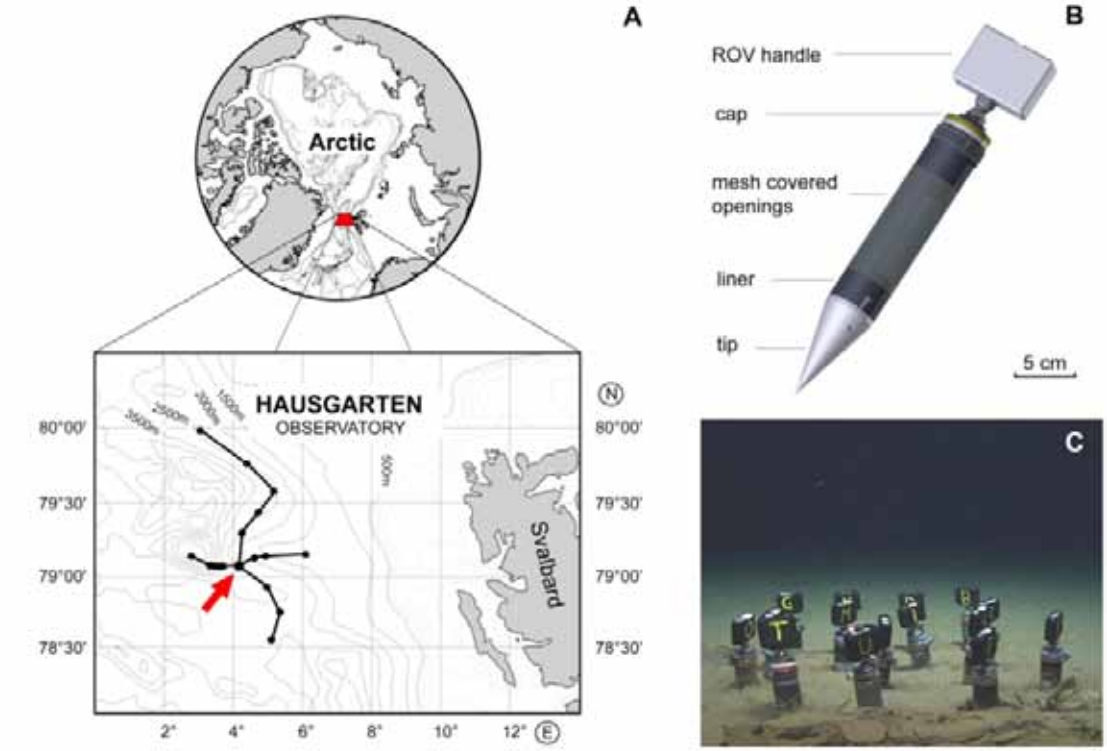
(H_5) Active migration is species-specific, i.e. some species may be more efficient than others and therefore, colonization might not be entirely random.

MATERIAL AND METHODS

Study site and sampling

The experiment was conducted at 2475 m water depth at the central HAUSGARTEN station (HG IV, Figure 1A) in the Fram Strait (west of Svalbard, 79°04.7′ N, 4°05.7′ E) during the ARK XXII-1c campaign in July 2007. HAUSGARTEN is a long-term observatory established in 1999 by the German Alfred Wegener Institute for Polar and Marine Research (Soltwedel et al. 2005). The remotely operated vehicle (ROV) *Quest* (MARUM, University of Bremen) carried out the video-controlled deployment and recovery of the experimental tubes. Reference samples, to determine the background nematode community composition, were collected from a single multi-corer (MUC) deployment conducted in close vicinity to the experimental site (July 2007, 79°03.9′ N, 4°10.6′ E, 2.2 km from the experimental plot). Three (pseudo) replicates were subsampled with syringes (2 cm diameter), sliced per centimetre down to a depth of 5 cm and fixed in 4% formalin. One push core from the ROV operations was taken, sliced per 5 cm to a depth of 15 cm and stored frozen at -20 °C to determine the background nematode $\delta^{13}C$ values, total organic carbon content (TOC) in the sediment and grain-size distribution. The sediment from below 15 cm depth in a box corer served as azoic sediment to fill the experimental colonization tubes and was also sampled with syringes for background nematode abundance, grain-size information and TOC of the sediment (3-times 3 syringes grouped together; 2 cm diameter, 5 cm length).

Figure 1. Location of the study area at the Arctic Marginal Ice Zone in the Fram Strait (Greenland Sea), west of Svalbard; a detailed design of the colonization tubes and a close-up of the experimental site at 2475 m water depth. A: Bathymetric map of the study area, with a dot at every HAUSGARTEN station. The arrow marks the central HAUSGARTEN station (HG IV) where the experiment was conducted. B: Colonization tube design with indicative scale. C: The experimental plot at 2475 m water depth with randomly distributed colonization tubes. Image taken by ROV Quest. Image copyright by MARUM, University of Bremen.



Cultivation and ^{13}C enrichment of food sources

Prior to the experiment, the cosmopolitan marine, centric diatoms *Thalassiosira pseudonana* (centric, singular, cell radius 3 µm) and *Skeletonema costatum* (cylindrical, filamentous, cell radius 3.125 µm) were both cultured axenically in f2 medium (Guillard 1975) at 15 °C with a 12:12-h light-dark period. The axenic state was insured by using sterilised sand, autoclaved glass material and artificial seawater, and 0.2 µ filters on injection needles while working under a flow bench. Diatoms were enriched by adding 5 ml of a $NaH^{13}CO_3$ MilliQ solution (^{13}C, 99%, Cambridge Isotope Laboratories, 336 mg per 100 ml MilliQ H_2O) to 100 ml of the culture

medium. After approximately 14 days, the algal material was harvested by centrifugation (3500 rpm, 5 min) and rinsed 3-times with artificial seawater to remove remaining bicarbonate ^{13}C from the algal suspension. The axenic state of the algae was verified by epifluorescence microscopy. This labeling technique resulted in an average $\delta^{13}C$ value of 43,259 ± 1,920‰ (equalling 33% ^{13}C) and 55,311 ± 675‰ (equalling 39% ^{13}C) for *T. pseudonana* and *S. costatum*, respectively.

In order to mimic a degrading algal bloom with an associated microbial community, *T. pseudonana* was also cultured non-axenically (i.e. non-sterilised conditions) under similar cultivation conditions as described above, but instead non-labeled $NaHCO_3$ was added to the f2 medium. To stimulate the growth and isotopically enrich the bacteria in the non-axenic culture, the algae were placed in the dark after 14 days of incubation and both unlabeled D-glucose and labeled ^{13}C-glucose (99% C6, Cambridge Isotope Laboratories) were added at 2.9 and 11.6 mg l^{-1}, respectively. After four days the mix of degraded algae and bacteria was extracted from the medium and washed in the same way as described for the axenic algae cultures. Average $\delta^{13}C$ of the labeled bacteria/algae mixture was 16,883 ± 1,949‰ (equalling 11% ^{13}C).

Benthic bacteria were inoculated from coastal sediments and cultivated in a modified M63 medium (Miller 1972) based on sterilized artificial seawater (salinity of 35 psu). Five g l^{-1} of glucose was added of which half was ^{13}C-labeled. After four days the bacteria were collected by centrifugation and washed 3-times before storing to remove excess ^{13}C-glucose from the bacterial suspension. An average $\delta^{13}C$ value of the bacteria was 70,991 ± 1,528‰ (equalling 44% ^{13}C).

The four cultured food sources were brought in suspension with artificial seawater, mounted in PVC spheres (4.4 cm diameter, 0.5 cm height, closed at the bottom with parafilm), and kept frozen at -20 °C until experimental use. This procedure produced thin frozen slices that could be easily introduced in the experimental tubes while even distribution of the food source was ensured. The carbon content in each slice was determined based on the amount of carbon per litre of culture and equalled 100 mg organic C m^{-2}. Due to the lack of information on carbon deposition at the HAUSGARTEN site at the time of the experimental set-up, the carbon concentration was chosen to correspond to the maximum carbon deposition per day calculated for the nearby, seasonally ice-covered East Greenland continental margin (10.9 mg C m^2 d^{-1}, Sauter et al. 2001), adapted to the duration of 10 days of the experiment. Meanwhile it has been shown that both areas have comparable seasonal flux patterns and amounts of settled organic matter over the year (Bauerfeind et al. 2009).

Experimental tube design and sediment preparation

The experimental tube design was inspired by Zhou (2001). The tubes were made from PVC cylinders (4.4 cm diameter) with 500 µm mesh covering the approximately 80% open contour over a length of 15 cm (Figure 1B). The 500 µm mesh was used to provide direct lateral access to nematodes dwelling in the surrounding sediments. The open cylinders were filled with sediment up to 2 cm from the upper mesh rim by pushing them into box corer sediment after the upper 15 cm of sediment was removed. The bottom of the tubes was closed with a conical cap which eased later insertion in the sediment. About 3 hours prior to deployment the frozen slices with ^{13}C labeled food sources were added on top of the sediment and covered with a few millimetres of sediment before the tubes were taped with parafilm to prevent the sediment from desiccating. The upper lids that closed the tubes had a handle that could easily be manipulated by the ROV *Quest* during deployment and recovery.

In-situ colonization / pulse-chase experiment

A total of 15 experimental tubes were prepared: three control tubes contained bare sediment, and 4-times three tubes contained sediment with one of the initially frozen ^{13}C-labeled organisms added on top. The tubes were stored in vials filled with seawater and mounted in a rack attached underneath the ROV Quest. At the deep-sea bottom (2475 m water depth) the tubes were handled one by one and inserted in the sediment while taking care that the manipulation would not flush sediment out of the tubes through the mesh. This was visually confirmed during placement and retrieval and later on-board while slicing the sediment. The tubes were randomly distributed in an area covering approximately 1 m² and positioned regularly spaced from one another (± 15 cm) (Figure 1C). On July 11th, the tubes were pushed into the bottom down to the level of the sediment in the tubes, leaving more or less 2 cm of the mesh open for water circulation. After 10 days of incubation, on July 20th, the tubes were retrieved undisturbed from the sediment by the ROV *Quest* and brought back to the surface. On board, the upper lid and lower pointed screw cap were removed and the sediment from the tubes was sliced in 1 cm slices down to 5 cm and one slice from 5 to 10 cm. Sediment slices were stored at -20 °C until further analysis in the laboratory.

Sample processing and analytical procedures

Grain-size distribution from one push core subsample (0 - 5 cm) and 3 box corer subsamples (15 - 20 cm) was measured using a Coulter Counter LS 100^{TM} Particle Size Analyser and classified according to Wentworth (1922). Sediments from a second series of subsamples were freeze-dried, homogenized and acidified with diluted HCl (1%) until all carbonates were

eliminated, before total organic carbon (TOC) was measured using a Carlo Erba elemental analyser.

Nematodes were extracted from the samples by triple density centrifugation with the colloidal silica polymer LUDOX TM 40 (Heip et al. 1985) and rinsed with tap water. All nematodes that passed through a 1000 µm mesh and were retained on a 32 µm mesh were counted and handpicked with a fine needle. Due to the low number of colonizers in the experimental tubes, both the community and the ^{13}C stable isotope analysis could not be performed on the three replicates from each treatment and control. It was decided to use one sample for nematode community analysis and two samples for measuring $\delta^{13}C$ values. Nematodes from different centimeter layers had to be combined for the ^{13}C analysis to ensure that sufficient biomass was available for EA-IRMS analysis.

All nematodes extracted from the three reference MUC samples, from the four experimental treatments and the control (one replicate each), and from the background box corer samples, were stained with Rose Bengal and transferred to De Grisse I, II and III (Seinhorst 1959) before being mounted on glass slides. Nematodes were identified to genus level. Adults were distinguished from juveniles based on the development of a vulva and uterus in females and a gonad and spicules in males. Nematode length (L, filiform tail excluded) and maximal width (W) were measured using a Leica DMR compound microscope and Leica LAS 3.3 imaging software. Nematode biomass was then calculated with Andrassy's formula (Andrassy 1956): wet weight (µg) = L (µm) x W^2 (µm) / 1.6 10^6, and a dry-to-wet-weight ratio of 0.25 was assumed (Heip et al. 1985).

The nematodes from the two remaining tubes per experimental treatment and control and from the push core (3-times 200 specimens) were rinsed in MilliQ water and transferred to a drop of MilliQ water in 2.5 × 6 mm, preglown aluminium cups at 550 °C in order to remove all exogenous organic carbon. Cups with nematodes and ^{13}C-labeled organic substrates were oven-dried at 60 °C, pinched closed and stored in multi-well Microtitre plates under dry atmospheric conditions until analysis. An elemental analyser-isotope ratio mass spectrometer (EA-IRMS; UC Davis Stable Isotope Facility, California; VUB, Brussels) was used to measure the carbon stable isotope ratios and carbon content. During this procedure a minimal He dilution was applied because of the low biomass of the nematode samples. Stable isotope ratios are expressed in the δ notation with Vienna Pee Dee Belemnite (VPDB) as reference standard, and expressed in units of ‰, according to the standard formula $\delta^{13}C = [R_{sample} / R_{VPDB} - 1] \times 10^3$, where R is the ratio of $^{13}C/^{12}C$ ($R_{sample} = [(\delta^{13}C_{sample} / 1000) + 1] \times R_{VPDB}$) and R_{VPDB} is 0.0111802. Label uptake by the nematodes is reflected as enrichment in ^{13}C and is presented as $\Delta\delta^{13}C$ (‰), which indicates the increase in $\delta^{13}C$ of the sample, as compared to its natural background value, and is calculated as

$\Delta\delta^{13}C$ (‰) = $\delta^{13}C_{sample}$ - $\delta^{13}C_{background}$. Hence, positive $\Delta\delta^{13}C$ values indicate that the organisms have acquired some of the introduced label. Absolute uptake of the label (I) is expressed in µg ^{13}C m^{-2} and calculated as I = (F_{sample} – $F_{background}$) x S, where F is the ^{13}C fraction F= R/(R+1) and S is the total carbon stock (µg C m^{-2}) of the nematodes. Biomass-specific respiration rates (R, d^{-1}) were based on the de Bovée and Labat formula (de Bovée and Labat 1993), corrected for in-situ temperature (-0.8 °C) assuming Q10 is 2: $R = 0.0449 \times W^{-0.1456} \times \exp^{\ln(Q10)/10(T-20)}$, where T is temperature (°C) and W is mean individual dry weight (µg C ind^{-1}). The individual carbon content was directly inferred from the carbon content measured by the EA-IRMS divided by the number of nematodes in a sample cup.

Data analysis

Nematode assemblages from the experimental treatments and the reference samples were analysed using univariate and multivariate techniques. One-way ANOVA was applied to assess differences between the univariate variables calculated for each sample (total nematode abundance, number of genera and Shannon-Wiener (H', $\log_e$) index). Total nematode abundances were initially logarithmically transformed to fulfil the assumption of homogeneity of variance. The post hoc Tukey HSD test was conducted where the one-way ANOVA obtained significant differences. The nonparametric Mann-Whitney U test was applied when relative abundance data of juveniles did not fulfil the homogeneity of variances test, and was used to test for differences between the colonizing and reference nematode assemblages. Non-metric Analysis of Similarities (ANOSIM) was used to test for significant differences between the nematode assemblage structure of the different experimental treatment samples and the experimental treatment samples versus the reference samples. The analysis was done on Bray-Curtis distances calculated from standardised and log(x+1) transformed abundance data. To visualise the multivariate structure of the nematode genera assemblages, non-metric multi-dimensional scaling ordination (MDS) was performed based on the same Bray-Curtis similarity matrix as used for ANOSIM. The variability among groups of samples was additionally analysed using the multivariate index of dispersion (MID) (Warwick and Clarke 1993). To determine the contribution of individual genera to the average Bray-Curtis dissimilarity between the experimental samples and the reference samples, the similarity percentages analysis (SIMPER) was applied to nematode relative abundances.

Nematodes were pooled into biomass and morphometric classes (length, width and length/width) on untransformed geometric scales. Biomass size spectra were created by plotting nematode cumulative relative abundances versus the biomass classes, while for creating the morphometric classes, nematode relative abundance were plotted against geometric classes of length (µm), width (µm) and length/width. All nematode measurements were therefore

pooled together in three groups (reference 0 - 5 cm, colonization 0 - 5 cm and colonization 5 - 10 cm). A chi-square (χ^2) goodness-of-fit test was performed to test whether the distributions of nematodes in biomass and morphometric classes in the experimental samples differed significantly from the reference samples.

All univariate analyses were performed using the software package STATISTICA 7, considering a confidence level of 0.05 in all tests (Statsoft Inc 2010). The multivariate analyses were carried out using the PRIMER v6 software package (Clarke and Gorley 2001; Clarke and Warwick 2001). Results are expressed as mean values ± standard deviation of replicates.

RESULTS

Sediment characteristics

Sediments from the push core (0 - 5 cm) and the box corer (15 - 20 cm) were very fine and characterised by a predominance of silt and clay. The upper 5 cm of the sediment in the push core differed from the 15 - 20 cm in the box corer by a higher clay and silt content (clay: 22% vs. 37 ± 1.2%, silt: 47% vs. 53 ± 2.3%, respectively) and a lower sand fraction (32% vs. 10 ± 1.9%, respectively) in the deeper sediment. The total organic carbon content was 0.7% and 0.4%, respectively.

Natural nematode community density and structure

The average nematode density over 5 cm depth in the reference samples was 1223 ± 365 ind. $10cm^{-2}$. The majority of the nematodes occurred in the top first centimetre with 45 ± 6.7% and gradually decreased downwards. A total of 56 genera were identified in the reference samples. The five dominant genera (relative abundance > 5%) altogether accounted for 49% of the total community (*Monhystrella*: 15.7%, *Thalassomonhystera*: 14.6%, *Tricoma*: 6.7%, *Acantholaimus*: 6.2%, *Halalaimus*: 5.9%).

Nematodes were only found occasionally in one of the three syringe samples taken from the box corer sediment below 15 cm depth (two *Thalassomonhystera* individuals). Their presence can therefore be considered as negligible and does not interfere with the colonization results. From now on the sediment that was used to fill the colonization tubes will be referred to as 'azoic sediment'.

Colonizing nematode community density, structure and diversity

After 10 days of incubation, mean nematode abundance in the first 5 cm of the colonization cores reached a maximum of 2.13% (51 ± 34 ind. 10 cm^{-2}) of the mean nematode abundance from the reference samples (Figure 2A). Because of the generally low and highly variable numbers of nematodes with depth, data from the first 5 cm were grouped for further analysis. As such, nematode abundances did not differ significantly, neither between the different experimental treatments (enriched) and controls (unenriched), nor between the upper and the lower 5 cm within the colonization tubes (Figure 2A, Appendix A). Compared to the reference, both layers of the colonized sediments contained a higher mean proportion of juveniles, with 74 ± 6% in the 0 - 5 cm and 75 ± 13% in the 5 - 10 cm, compared to 58 ± 4% in the reference samples. Only the difference between the top 5 centimetres was significant ($p = 0.025$, Appendix B).

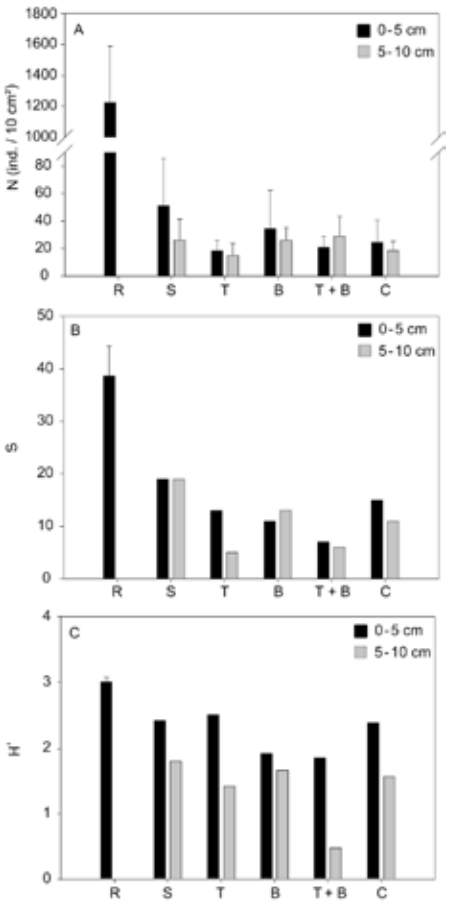
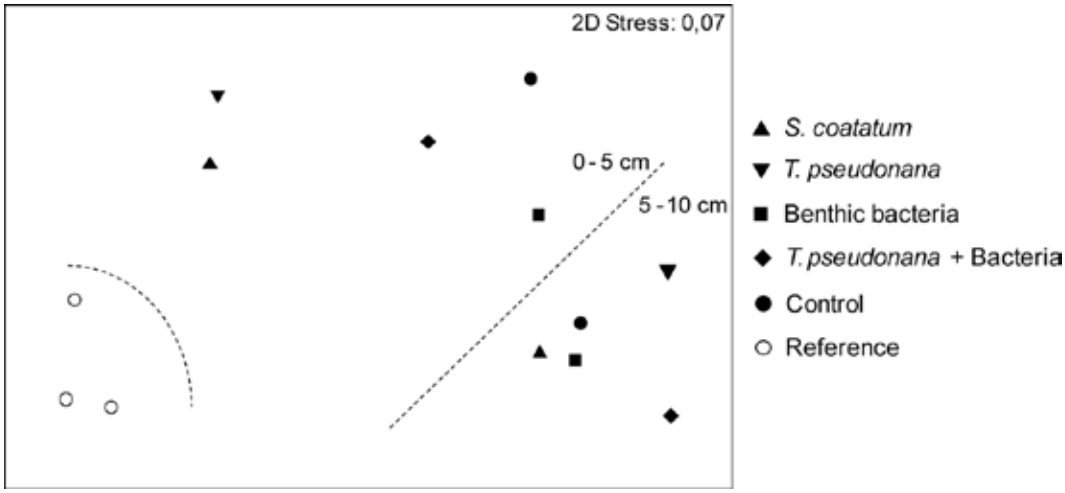


Figure 2. Density (N) and diversity (S, H') of the nematode assemblages in the reference sediments and all five experimental treatments. Mean and standard deviation where possible for (A) nematode density (N, ind. 10cm^{-2}), (B) number of genera (S) and (C) Shannon-Wiener diversity index (H', log_e) in the reference samples (R) and the different experimental treatments (S: Skeletonema costatum, T: Thalassiosira pseudonana, B: benthic bacteria, T + B: Thalassiosira pseudonana + bacteria) and control (C) samples; in the 0 - 5 cm (black bars) and 5 - 10 cm (grey bars) sediment layers.

A pairwise ANOSIM test showed that nematode assemblages in all enriched and unenriched experimental tubes differed in genus composition from the references ($0.75 \leq R \leq 1$), but did not differ from one another ($R \leq 0.5$) (Figure 3, Appendix C). Furthermore, a small though significant differentiation in nematode assemblages was found among the experimental tubes according to depth in the sediment ($R = 0.588$, $p = 0.008$, Appendix C). The upper and lower 5 cm intervals will further be treated separately to allow comparison with the reference samples which comprise only the upper 5 cm of the sediment. As the different enriched experimental treatments and the unenriched controls did not have a significantly different effect on the nematode assemblage that colonized the tubes, they were considered as one group for further description and analysis of the colonizer assemblage structure. The multivariate index of dispersion (MID) showed that the variability is relatively high among the experimental tubes (0 - 5 cm: 1.467, 5 - 10 cm: 0.692) compared with the references (0 - 5 cm: 0.472).

Figure 3. Non-parametric multi-dimensional scaling for nematode assemblages from the references and the experimental controls and enriched treatments (Skeletonema coatatum, Thalassiosira pseudonana, benthic bacteria and bacteria grown on degrading T. pseudonana). The dotted lines separate the samples according to significant ANOSIM grouping results ($R \geq 0.588$, $p \leq 0.008$).



A total of 41 genera colonized the azoic sediments, of which only 29 were also found in the references. The number of genera found in both the upper and lower 5 cm sediment in the colonization tubes are also significantly lower than in the references ($p \leq 0.0003$, Appendix A) (Figure 2B). In the upper 5 cm of the sediment 33 genera were found, while in the lower 5 cm horizon 26 genera were found (7 and 8 genera were not found in the reference samples, respectively). Colonizers that were exclusively found in the colonization tubes were rare and never occurred with a mean relative abundance higher than 1.5%. Genera that dominate colonization in the top 5 cm (mean relative abundance > 5%) altogether accounted for 67.6% of the total colonization assemblage (*Sabatieria*: 16.5%, *Thalassomonhystera*: 15.4%, *Leptolaimus*: 12.6%, *Anoplostoma*: 11%, *Dichromadora*: 6%). These four genera were also found in the reference samples (together representing 25%), with only *Thalassomonhystera* as mutually dominant genus. The dominant genera from the reference samples are represented by 21% in the colonizing community and genera that can be considered as rare in the reference samples (< 1.5% relative abundance) make up 35% of the colonizing assemblage in the top 5 cm. In the lower 5 cm of the colonization tubes, *Sabatieria* was the only dominant genus and accounted for 68.2% of the colonizing specimens. Diversity in terms of the Shannon-Wiener index (H') was also significantly higher in the reference samples (3.0 ± 0.08) compared to both depth layers of the colonization tubes ($p \leq 0.05$, Appendix A). Among the colonization tubes the upper 5 cm of the sediment (2.2 ± 0.31) are characterised by a higher diversity compared to the lower 5 cm (1.4 ± 0.53) ($p = 0.017$, Appendix A) (Figure 2C).

Results from the SIMPER analysis based on the relative abundance data are complementary to the ANOSIM and MID results and revealed that differences between the references and both depth layers of the colonized azoic sediments were mainly due to higher proportions of *Sabatieria* in the colonized sediments. *Sabatieria* accounted for 12% and 34.6% of the total dissimilarity between the references and the upper 5 cm and the lower 5 cm of the colonization tubes, respectively. The genus *Anoplostoma* had a relatively high abundance in the upper 5 cm of the colonization tubes and contributed as such with 10.2% to the dissimilarity with the references. *Monhystrella* and *Thalassomonhystera* both accounted for > 5% of the dissimilarity between the reference and the colonized sediments, by occurring in higher relative abundances in the references (Table 1).

Table 1: Results from a similarity percentage (SIMPER) analysis, indicating % of similarity and dissimilarity between the nematode assemblages from the reference and the colonization tubes, with discriminating genera contributing to > 5% of the (dis)similarity.

	Reference (0 - 5 cm)		Colonization tubes (0 - 5cm)		Colonization tubes (5 - 10 cm)	
Reference (0 - 5 cm)	**similarity**	**66.5**				
	Monhystrella	19.3				
	Thalassomonhystera	18.4				
	Acantholaimus	7.8				
	Tricoma	7.7				
	Halalaimus	5.3				
Colonization tubes (0 - 5cm)	**dissimilarity:**	**70**	**similarity:**	**43.1**		
	Sabatieria	12	*Sabatieria*	27.2		
	Anoplostoma	10.2	*Anoplostoma*	24.3		
	Monhystrella	9.8	*Dichromadora*	13.7		
	Thalassomonhystera	5.9	*Thalassomonhystera*	12.1		
			Campylaimus	7.3		
			Leptolaimus	6.7		
Colonization tubes (5 - 10 cm)	**dissimilarity:**	**84.9**	**dissimilarity:**	**64.3**	**similarity:**	**62.2**
	Sabatieria	34.6	*Sabatieria*	32.8	*Sabatieria*	86.4
	Monhystrella	8.3	*Anoplostoma*	9.4		
	Thalassomonhystera	6.8	*Thalassomonhystera*	8.1		

δ^{13}C stable isotope values

The natural δ^{13}C signal derived from 3-times 200 nematode individuals (approximately 10 µg C) from the top 5 cm in the reference push core averaged -18.08 ± 0.3‰. Label uptake by colonizers was traced in bulk nematodes from the upper five and lower five centimetres separately. Due to the low number of nematodes that colonized the experimental tubes, the reliable detection limit of 8 - 10 µg C was often not reached, despite the higher biomass of a large fraction of the colonizers. Therefore, isotope ratios based on a carbon amount lower than 5 µg C were discarded. The specific ^{13}C label uptake ($\Delta\ \delta^{13}$C) by the colonizing nematodes reached a maximum of 7.31‰ in the treatment where bacteria grown on *Thalassiosira pseudonana* diatoms were offered (Table 2). This equals an absolute uptake (I) of 0.019 µg C tube^{-1} or 0.012% of the added carbon. Based on the formula of de Bovée and Labat (1993) for biomass-specific respiration estimates, this corresponds to only 2.8% of carbon respired over 10 days. Although poor replication due to the low number of colonization hampered statistical

analysis, it is clear that in none of the experimental treatments nematodes fed significantly on the labeled potential food sources. This supports the findings on nematode abundances that were not higher in the food treatments compared to the unenriched treatment, indicating that the added food sources were not the trigger for nematode colonization.

Table 2. $\delta^{13}C$ (‰) values of the enriched treatments, based on 10 to 88 nematode individuals per analysis. Replicates are indicated by A and B. Values obtained from less than 5 µg C are left out. Nematode reference $\delta^{13}C$ values: -18.08 ± 0.3‰ (based on 200 nematode individuals, 3 pseudo-replicates).

		S. costatum		***T. pseudonana***		**Benthic bacteria**		***T. pseudonana* + Bacteria**	
		A	B	A	B	A	B	A	B
0 - 5 cm	**µg C:**	6.44	6.47	4.90	/	/	13.53	/	/
	$\delta^{13}C$ (‰):	-15.04	-17.69	-15.40	/	/	-16.30	/	/
5 - 10 cm	**µg C:**	8.73	9.35	7.78	/	12.29	11.05	5.26	8.52
	$\delta^{13}C$ (‰):	-14.60	-18.83	-19.12	/	-17.72	-17.06	-10.77	-16.14

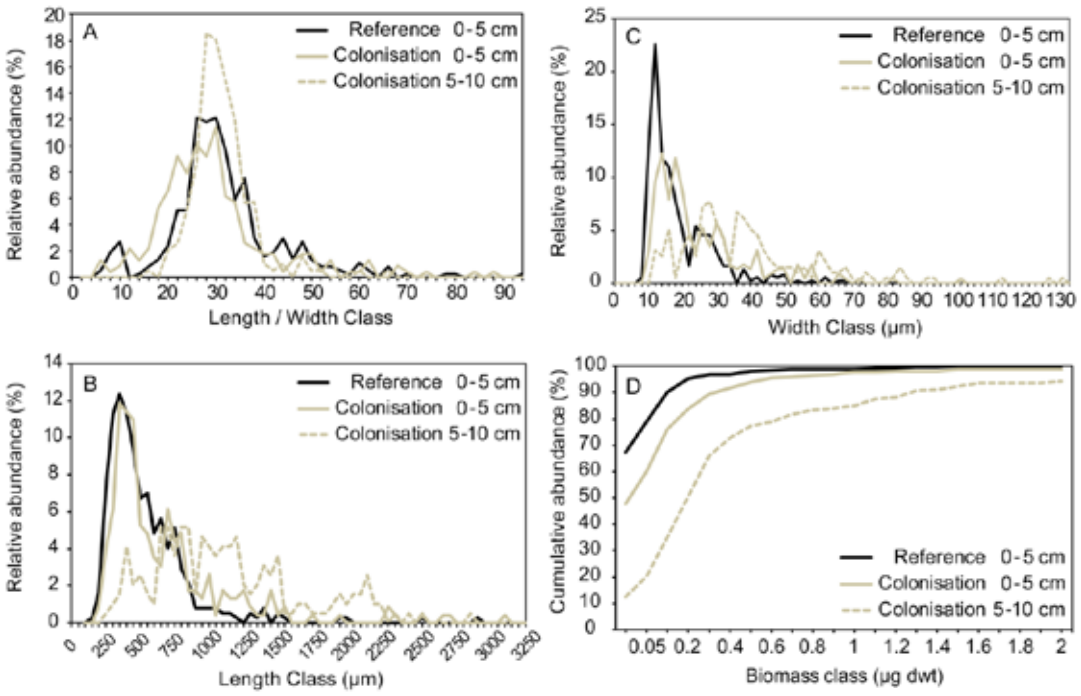
Nematode morphometry and biomass

Body measurements (length and width) were done on the complete nematode assemblage of the reference and colonization samples (Figure 4). The distribution of nematode morphometric classes in the reference sediments showed a bimodal shape in case of the length/width ratio, indicating two distinct morphological groups (Figure 4A). These two morphotypes are visualized as two peaks, at L/W ratios of 8 - 10 and of 24 - 30, with a distinct minimum at a ratio of 10 to 14, reflecting short, stout nematodes and more slender, on average longer nematodes, respectively. The morphometric class distribution in both 5 cm depth layers of the colonized sediments shifted from the reference distribution in a different way but lacked this distinct group of short, stout morphotypes, represented by the genera *Desmoscolex* and *Tricoma*, in common. In the upper 5 cm of the colonized sediments there was a relatively large shift towards greater body width compared to the shift towards greater body length, resulting in more plump nematodes (Figure 4A-C). The more equally spread distribution in length and width of the colonizers in the 5 - 10 cm sediment depth led to a narrower spectrum and indicates more slender morphotypes are present (Figure 4A-C). In the reference samples, 20% of the nematodes have a L/W ratio < 24, while in the experimental samples the shift is clear towards 37% in the upper 5 cm and towards 10% in the lower 5 cm of the sediment.

Nematodes assemblages from the 5-10 cm colonized sediments were characterized by a greater body length and body width compared to both the reference and upper 5 cm of colonized sediments (χ^2 test, $p \leq 0.0006$, Appendix D).

In the reference samples, 95% of the nematode assemblage consisted of individuals with a biomass of less than 0.2 µg dry weight (Figure 4D). This biomass class represented 84% in the upper 5 cm and only 51% in the 5 - 10 cm in the colonized sediment. A chi-squared (χ^2) analysis showed that colonization of the 5 - 10 cm sediments was characterized by nematodes with higher biomass, compared to the upper 5 cm of the colonized sediments and the reference sediments ($p \leq 0.00001$, Appendix D).

Figure 4. Relative abundance of nematodes in geometric and biomass classes for the reference and experimental colonization samples, with indication of the respective depth (0 - 5 cm, 5 - 10 cm). (A) Length/Width geometric classes (µm), (B) Length geometric classes (µm), (C) Width geometric classes (µm) and (D) Biomass classes (µg dry weight). The relative abundances are based on 372, 228 and 194 individual measurements in the reference, the 0 - 5 cm colonization and the 5 - 10 cm colonization category, respectively.



DISCUSSION

Studies on the response of deep-sea nematode communities to organic matter pulses generally focussed on changes in species composition and biomass patterns, or feeding ecology by means of simulated phytodetritus fluxes with ^{13}C-labeled algae or bacteria. Our study differs by investigating the reaction of deep-sea nematodes on ^{13}C-labeled organic matter in terms of colonization and uptake. Although conducting experiments in situ in the poorly accessible deep sea, using ROV technology, renders very precise control over the experiment, it also comes at a cost. The limited storage space on the ROV, in combination with limited ship time, restricted replication of the experimental treatments and only allowed sampling at one moment in time. Replication was further restricted by the low number of colonizing nematodes. Nonetheless, our experimental set up allowed simulating disturbance and the arrival of different types of organic matter in situ, with minimal manipulation or influence on the natural nematode community.

Colonization rates

The infaunal colonization of azoic sediments observed in our experiment confirms the first hypothesis (H_1) that deep-sea nematodes can actively colonize open patches. It affirms the findings of Gallucci et al. (2008b) that deep-sea nematodes have the ability to respond within a time frame of a few days (or less). The colonization rates in the upper 5 cm of our experimental tubes (max. 51 ind. 10 cm^{-2}, i.e. 2% of the reference) are however lower than reported for infaunal migration of coastal nematodes, where nematodes relative densities reached 4% - 36% after 2 days and 19% - 31% after 29 days (Chandler and Fleeger 1983); 4% - 7% after 10 days (Zhou 2001); and 32% - 90% after 14 days (Schratzberger et al. 2004) of the reference nematode abundances. Gallucci et al. (2008b) used a comparable setup with the upper 5 cm sediment from 1300 m of water depth in an on-board microcosm experiment and found colonization rates of approximately 5% - 20% after 9 days, without significant difference after 17 days. These rates are similar to or at the lower end of the rates found for coastal sediments, but higher than reported in the present study. In case where food was added to the sediment, the higher concentrations of added organic carbon in the experiment of Gallucci et al. (2008b) might have induced higher colonization rates (see below) (Zhou 2001). Nonetheless, the colonization rates in the unenriched controls in this study are also comparatively low. Since Gallucci et al. (2008b) used deeper sediment layers (below 8 cm sediment depth) as colonization substrate, it is unlikely that the use of slightly deeper sediment layers in this study (below 15 cm sediment depth) is responsible for the observed differences in colonization rates between the two studies. A factor with unknown impact is, however, the effect of decompression prior to the laboratory experiment of Gallucci et

al. (2008b). In our study the colonized sediments contained a relatively small higher fraction of clay and silt compared to the surrounding sediment. Whether this might have introduced a barrier or perhaps less favourable conditions for nematodes to colonize is unclear. Only the effect of relatively coarser sediments on nematode colonization was tested before (Schratzberger et al. 2004). Differences in colonization rates in intertidal coastal sediments were only significant in the treatment where sandy sediments (mud, < 63 µm: 9.3 ± 0.6%, sand: 90.4 ± 0.8%) were inserted in mud (mud; < 63 µm: 92.6 ± 3.3%, sand: 7.4 ± 3.3%) collected from the field (Schratzberger et al. 2004). Since the relative difference in sand and mud fractions between the colonization and reference sediment in this study is only relatively small (sand: 10% versus 31%, mud: 90% versus 69%, respectively), the grain size distribution per se probably didn't influence the colonization rates. Nevertheless, there are still other covarying sediment parameters that we did not measure, but may have had an effect (e.g. porosity, pH, O_2 concentration). These uncertainties withhold us from supporting the debated suggestion on slower colonization rates in the deep sea (Grassle 1977; Gage 1991).

When compared to the upper 5 cm of the colonized sediments, nematodes occurred in equal abundances and number of genera, below 5 cm in all experimental treatments. The nematode assemblage that colonized the top 5 cm of the experimental tubes was however more diverse due to the dominance (with 68%) of *Sabatieria* in the lower 5 cm of the colonization tubes. Overall, the total number of genera that colonized the azoic sediments in our short-term experiment was relatively high (41 genera) and only slightly lower compared to the reference situation (56 genera). Together with the high variability between the colonizing assemblages, our results confirm that early deep-sea colonization of disturbed patches is a highly localised process, with reproducible abundance and diversity patterns, but a relatively poor predictability of the community composition (Gallucci et al. 2008b).

The role of organic matter in the colonization process

Nematode assemblages colonized the azoic sediments in the enriched and the unenriched experimental tubes in equal numbers and with similar diversity, irrespective of the presence or the type of organic matter added. Based on these observations the second hypothesis (H_2), that postulated that colonization rates are enhanced by the presence of food and led to higher abundances and number of genera, is rejected. This is in contrast with the experimental results of Gallucci et al. (2008b) who observed higher nematode abundances and number of nematode species in diatom enriched sediments compared to unenriched sediments after 9 days in a microcosm experiment performed at a shallower station at the Arctic HAUSGARTEN site. The four times higher nematode abundance in enriched compared to the unenriched sediments observed by Gallucci et al. (2008b) might be the result of

applying higher concentration of organic matter compared to this study (1 g vs. 100 mg organic C m^{-2}, respectively). The positive effect of higher concentrations of organic matter was found before in a colonization experiment in a mangrove (Zhou 2001). As a result of leaf litter addition significantly higher relative nematode numbers compared to the controls appeared after 30 days and showed a positive response to different levels of leaf litter addition after 60 days. This response was however mainly due to a single opportunistic, bacterivorous species (*Diplolaimella* sp.) that represented up to 84% of the colonizing assemblage, and was otherwise rare in unenriched and ambient mangrove sediments. In the study by Gallucci et al. (2008b), not one but several genera that were rare or undetected in the natural sediments all together dominated colonization of defaunated sediments, indicating a different reaction. In contrast to Gallucci et al. (2008b), the rare and undetected genera from the reference samples in our study only represented 35% of the relative abundance of the colonizing assemblage. If the suggested slower colonization rates in the deep sea hold true, our findings might reflect an earlier stage in the colonization process compared to Gallucci et al. (2008b).

Additionally, we could verify that the colonizing nematodes did not feed significantly on the different types of labeled food added to the experimental tubes. This also means a rejection of the third and fourth hypothesis (H_3 and H_4). While food is generally thought of as an attracting factor for free-living marine nematodes (e.g. Lee et al. 1977; Neira et al. 2001; Höckelman et al. 2004) in some cases it seems as if certain food varieties or stages of decomposition have a repellent or indifferent effect on some communities and species (Lee et al. 1977; Gooday et al. 1996; Zhou 2001). The indifferent effect of the presence of pelagic diatoms as observed in our experiment was also found under experimental conditions by Ullberg and Ólafsson (2003a). They suggested that nematodes from the shallow coastal station they sampled and incubated preferred in-situ growing diatoms above pelagic diatoms as they more closely resemble the natural circumstances. However, by using the cosmopolitan pelagic diatoms, benthic bacteria and bacteria grown on degrading pelagic diatoms (detritus) we covered the most relevant range of potential food sources that deep-sea nematodes have access to. Bacterial biomass is one to two orders of magnitude higher than nematode biomass in ocean basins worldwide (Rex et al. 2006) and, according to respiration estimations of a typical deep-sea nematode community, a theoretically non-limited potential food source (Guilini et al. 2010). Nonetheless, the results from this colonization experiment and from a ^{13}C-tracer experiment that directly labeled the natural deep-sea bacterial community at 1280 m depth at the HAUSGARTEN site, both suggest that on a short term bacterial carbon is of no substantial importance in the diet of the deep-sea nematode community (Guilini et al. 2010). On the other hand, organic matter enrichment by means of diatoms seemed especially relevant based on the fact that they are among the organisms that dominate the POM flux derived from phytoplankton blooms following the retreat of winter sea ice at high latitudes (maxima in

May - June and August - September) (Bauerfeind et al. 2009). Evidence of high-quality detritus rapidly reaching the deep-sea floor has been reported for numerous deep-sea sites (e.g. Billett et al. 1983; Lampitt 1985; Beaulieu 2002). Nevertheless, in terms of uptake, the results of this study support other recent ^{13}C stable isotope tracer experiments in deep-sea sediments, all indicating a limited contribution of nematodes to the short-term processing of labeled phytodetritus (Moodley et al. 2002; Witte et al. 2003b; Nomaki et al. 2005; Moens et al. 2007; Sweetman and Witte 2008; Ingels et al. 2010a, b).

One plausible explanation is that the experiment was performed at the same time or after a natural phytodetritus flux settled to the sea floor and nematodes were surrounded by or already processed a great amount of detritus, leaving them indifferent to the food we applied. Additionally or alternatively they might have a very specific preference for a certain component of the sediment or detritus flux that we did not address (e.g. coccolithophorids, pennate diatoms, ciliates, protists like tintinnids, foraminifera, radiolaria or acantharia; Bauerfeind et al. 2009). Forest et al. (2010) and Bauerfeind et al. (2009) however, recently illustrated that not more than 2% of the photosynthetically-produced carbon in the Fram Strait may reach ca. 300 m water depth due to a high degree of recycling within the upper water column. Fresh diatoms were negligible in the sediment trap samples and most of them were degraded frustules with no plasma content (Forest et al. 2010). These results indicate that coupling between pelagic-benthic fluxes might be overestimated in high-north latitudes. Nonetheless, high primary production at the ice edge results in high phytodetritus concentrations at the sea floor (Schewe and Soltwedel 2003) and nematode densities are correlated with water depth and pigment concentrations in the sediment at the HAUSGARTEN site (Hoste et al. 2007). Therefore, an alternative, though speculative, explanation for the lack of uptake might be that the combination of seasonal detritus fluxes, low-temperature induced slow nematode growth, long life span and low maintenance costs (Giere 2009), and low-temperature reduced microbial respiration at low concentrations of organic matter (Nedwell 1999), lead to an accumulation of labile organic material in Arctic deep-sea sediments. Similar to what is suggested for Antarctic shelf sediments by Mincks et al. (2005) and Smith et al. (2006) this could result in a persistent 'food bank' for benthic detrivores over long time scales relative to the seasonality of POC deposition. Additionally, if the success of deep-sea nematodes lies partly on trophic specialisation that implies unselective deposit feeding rather than active selection of food particles, as suggested by Giere (2009), nematodes might be less limited in food than is expected till now. This could enforce the possibility that the deep-sea nematode colonization processes in the Arctic area are not mainly driven by food-related features, as observed in our experiment, but that it is rather the result of attraction by biogeochemical cues that were unaccounted for or 'random walk' behaviour (whether or not driven by the search for a mating partner). However, the lack of data that cover seasonal variability

in concentrations of labile organic matter (reflected in e.g. chlorophyll a, enzymatically hydrolysable amino acids, etc.), as well as concentrations of POC and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of both settling POM and surface sediments at the Arctic ice margin, does not enable us to verify if the sediment has a buffering effect against water column variability, and the labile organic matter persists year-round, or not.

Colonizing efficiency

The nematode assemblages that colonized the azoic sediments differed significantly from the natural nematode assemblage. Some genera colonized more efficiently, thus supporting the fifth hypothesis (H_5) that active colonization is species-specific. The colonization success of certain *Sabatieria* and *Leptolaimus* species for example was emphasised by Gallucci et al. (2008b), Ullberg and Ólafsson (2003b) and Schratzberger et al. (2004), and is confirmed here. These observations indicate that in shallow- and deep-water environments species association upon initial colonization is not simply the result of random processes, but determined by species characteristics such as their motility and colonization ability (Horn 1981). While in the upper 5 cm of the colonized sediments five dominant genera (*Sabatieria*, *Thalassomonhystera*, *Leptolaimus*, *Anoplostoma* and *Dichromadora*) accounted for 68% of all nematodes encountered, *Sabatieria* alone dominated with 68% in the lower 5 cm. The success of some *Sabatieria* species has been attributed to their high tolerance to anoxia, relatively big size (adult average length in this study: 1918 ± 285 µm) and higher mobility which enables them to move through compacted subsurface sediments and easily access oxygen in the upper layers (Soetaert et al. 2002). While members of the genus are found in muddy sediments that are anoxic from just a few millimetres below the sediment surface, *Sabatieria* also occurs deeper down in more oxygenated sediments, in the vicinity of the redox-potential-discontinuity layer (Vanreusel 1990; Vincx et al. 1990; Hendelberg and Jensen 1993). Sachs et al. (2009) reported oxygen penetration at the central HAUSGARTEN station (2500 m water depth) down to 15 cm. Therefore it is feasible that *Sabatieria* lives below 5 cm where oxygen and also organic matter are limited, and regularly migrates upwards to access food. This was demonstrated for *Sabatieria* at a subtidal station in the southern North Sea by means of natural stable isotope signatures (Franco et al. 2008). Unfortunately, reference data of the nematode community for the HAUSGARTEN site are restricted to the upper 5 cm of the sediment. Nonetheless, *Sabatieria* is demonstrated to be among the most efficient colonizers, especially of deeper sediment layers. *Thalassomonhystera* on the other hand, belongs to the family Monhysteridae, whose reproductive rates are generally higher and development rates faster than for most other marine nematodes (Heip et al. 1985, Bongers et al. 1991). Having the characteristics of an r-strategist, it is curious that *Thalassomonhystera*'s dominance prevailed in later successional stages. In contrast, other dominant genera in the

reference samples: *Monhystrella* (family Monhysteridae), *Acantholaimus* and *Halalaimus*, were rarely encountered in the experimental tubes. These genera are characterised by a long filiform tail, which was suggested to be typical for a hemi-sessile life strategy (Riemann 1974). Although the size of *Acantholaimus* and *Halalaimus* does not necessarily imply limited active migration ability, as is suggested for the very small *Monhystrella* genus, their response to disturbance may as well involve a different strategy (e.g. high reproductive rates) and rely less on motility (Gallucci et al. 2008b). The remaining 32% of the total nematode assemblage that colonized the azoic sediments counted 36 genera of which twelve genera were exclusively found in the colonization tubes, never with a relative abundance higher than 1.5%. The absence of these rare genera from the reference samples might partly be a result of undersampling, as three reference (pseudo-)replicates underestimate the natural variability and genus richness of nematode assemblages on the small local scale of the experiment. This is confirmed by the fact that 7 of these 12 genera were encountered by Hoste (2006) in a 5 year time-series study across a depth gradient at the HAUSGARTEN site. On the other hand, the fact that 35% of the colonizing assemblage are rare or absent in the reference samples might also support and argue that a quick response to open space and/or organic matter deposition is an important life strategy to counterbalance local extinctions and inhabit a patchily disturbed environment (Gallucci et al. 2008b).

The distribution of nematode morphometric classes (L/W) in the reference sediments showed a bimodal shape. This is a typical and persistent feature found in nematode communities in continental slope areas around the world, indicating two distinct morphological groups (Soetaert et al. 2002). In the colonized sediments, the size spectrum shifted towards thicker nematodes in the upper 5 cm and longer and wider nematodes in the lower 5 cm, confirming the fifth hypothesis (H_5) and the experimental findings of Gallucci et al. (2008b) that active migration is dependent on nematode size. Our findings suggest two potential strategies. The thicker nematodes found in the upper 5 cm of the colonized sediments might be genera that allocate a larger amount of food to storage products rather than to structural growth. This could provide them with the advantage of remaining an increased amount of time without food, depending on their stored reserve products (Soetaert et al. 2002). On the contrary, the bigger individuals found in the lower 5 cm of the sediment most probably and perhaps additionally have the advantage of a high mobility, which provides accessibility to food and open space, and enables exchange between hypoxic subsurface and oxic surface layers on a short notice. The shift in nematode size towards bigger colonizers also co-occurs with a shift in developmental stages towards more juveniles in the colonizing assemblage. This confirms the suggestion of Soetaert et al. (2002) that if bigger nematodes have a higher age at maturity, the nematode communities that are, on average, composed of larger species would be more dominated by juveniles.

This statement is based on the assumption that smaller-sized species that have higher growth rates (biomass increase; Peters 1983) also have reduced age at first breeding in the deep sea (Soetaert et al. 2002).

Prospects

Caution should be taken when extrapolating the results to situations of medium- to large-scale disturbances. Not only can colonization processes vary markedly between patches of different sizes (Smith and Brumsickle 1989) or disturbance intensity, the process will also be more complex when it is influenced by water column processes such as bottom currents or sediment upwelling transporting nematodes (Ullberg and Ólafsson 2003a), or macrofaunal interstitial migration and larval settlement. This demonstrates that the understanding of local dynamics in the deep sea is still in its infancy. Meanwhile, the idea and associated modelling efforts arose on local communities being embedded in a metacommunity, where it is likely that various spatial dynamics alter local community responses that feed back to alter features of regional biota (Leibold et al. 2004). Therefore, if we want to (1) make progress in understanding deep-sea community ecology, (2) implement local dynamics in metacommunity models and (3) anticipate the impact of acute disturbances in the deep sea, it is required to gather autoecological information and perform prolonged in-situ experimental incubations with repeated sampling over time to follow up further successional stages and complete recovery time.

CONCLUSION

This study demonstrated that deep-sea nematodes actively colonized azoic sediments within a time frame of 10 days, regardless of the presence or the type of organic matter that was added. The fact that organic matter did not function as an attractant was confirmed by the absence of notable ^{13}C assimilation by the colonizing nematodes. Colonization by deep-sea nematodes appears to be a process yielding reproducible abundance and diversity patterns, with certain taxa showing more efficiency, partly based on body shape characteristics such as size and motility. Colonization by a relatively large number of genera that were rare or undetected in reference sediments also suggests that small-scale disturbance contributes to their persistence in deep-sea sediments. Together with the high variability between the colonizing nematode assemblages, these results lend experimental support to the existence of a spatio-temporal mosaic that emerges from highly localised, both stochastic and deterministic community dynamics.

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ADDENDUM

Appendix A. One-Way ANOVA test results for differences in nematode abundance, genus richness (S diversity) and Shannon-Wiener *diversity (H' diversity) between the experimental colonization tubes (Exp.) and the experimental colonization cores and the reference sediments (Ref.), respectively. (T: treatment, C: control)*

Main effect	SS	df	MS	F	p	Post-hoc Tukey test	p
Abundance	534.14	3	178.05	0.617	0.609	Exp. T (0-5 cm) vs. Exp. T (5 10 cm)	0.682
						Exp. T (0-5 cm) vs. Exp. C (0-5 cm)	0.957
						Exp. T (0-5 cm) vs. Exp. C (5-10 cm)	0.717
						Exp. T (5-10 cm) vs. Exp. C (0-5 cm)	0.999
						Exp. T (0-5 cm) vs. Exp. C (5-10 cm)	0.976
						Exp. C (0-5 cm) vs. Exp. C (5-10 cm)	0.973
S diversity	1624.53	2	812.27	33.64	0.00004	Exp. (0-5 cm) vs. Exp (5-10 cm)	0.765
						Exp. (0-5 cm) vs. Ref. (0-5 cm)	0.0003
						Exp. (5-10 cm) vs. Ref. (0-5 cm)	0.0002
H' diversity	5.09	2	2.55	17.034	0.0006	Exp. (0-5 cm) vs. Exp (5-10 cm)	0.017
						Exp. (0-5 cm) vs. Ref. (0-5 cm)	0.046
						Exp. (5-10 cm) vs. Ref. (0-5 cm)	0.0007

Appendix B. Mann-Whitney U test results for differences in relative abundances of juveniles between both depth layers of the experimental colonization tubes (Exp.) and the reference sediments (Ref.).

Effect	U	Z adjusted	p
Exp. (5-10 cm) vs. Ref. (0-5 cm)	2	1.6	0.101
Exp. (0-5 cm) vs. Ref. (0-5 cm)	0.0	2.24	0.025

Appendix C. ANOSIM test results on differences in nematode community composition between the different experimental treatments (Exp.) and the reference sediment (Ref.), and between the different sediment depth layers in the experimental treatments (Exp.) and the reference sediments (Ref.). (S: Skeletonema costatum, T: Thalassiosira pseudonana, B: benthic bacteria, T + B: Thalassiosira pseudonana + bacteria, C: control).

Factor Main test	**Global R**	**p**	**Factor Pairwise test**	**R**
Exp. vs. Ref.	0.554	0.008	S vs. T	0
			S vs. B	0.25
			S vs. T+B	0.25
			S vs. C	0.5
			S vs. Ref.	0.75
			T vs. B	-0.25
			T vs. T+B	-0.5
			T vs. C	0
			T vs. Ref.	0.75
			B vs. T+B	0
			B vs. C	0.5
			B vs. Ref.	1
			T+B vs. C	0.25
			T+B vs. Ref.	1
			C vs. Ref.	1
Sediment depth	0.676	0.001	Exp. (0-5 cm) vs. Exp. (5-10 cm)	0.432
			Exp. (0-5 cm) vs. Ref. (0-5 cm)	0.682
			Exp. (5-10 cm) vs. Ref. (0-5 cm)	0.969

Appendix D. χ^2 Goodness of fit test results for differences in nematode body length, width and biomass between the experimental colonization tubes (Exp.) and the reference sediments (Ref.).

Tested effect	**Factor**	**chi-square**	**df**	**p**
Body length	Ref. vs. Exp. (0-5 cm)	26.06	75	1
	Ref. vs. Exp. (5-10 cm)	261.13	48	0.00001
	Exp. (5-10 cm) vs. Exp. (0-5 cm)	221.54	75	0.00001
Body width	Ref. vs. Exp. (0-5 cm)	72.16	64	0.23
	Ref. vs. Exp. (5-10 cm)	222.46	64	0.000001
	Exp. (5-10 cm) vs. Exp. (0-5 cm)	107.25	64	0.0006
Biomass	Ref. vs. Exp. (0-5 cm)	6.82	48	1
	Ref. vs. Exp. (5-10 cm)	118.94	48	0.00001
	Exp. (5-10 cm) vs. Exp. (0-5 cm)	136.23	48	0.00001

4

Evidence for the trophic state and bentho-pelagic coupling of deep-sea nematodes across the Southern Ocean

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ABSTRACT

Nematodes are key organisms among deep-sea benthos. To understand the processes that shape nematode communities in the deep Southern Ocean, structural and functional feeding aspects of nematodes collected during the ANT XXIV-2 (2007/08) expedition were investigated in relation to environmental variables. Samples were collected from six stations along the Prime Meridian (49° S - 70° S), including a repeated sampling after one and a half months interval at a Polar Front station (52° S), to elucidate the short-term response on a seasonal deposit of detritus. Nematode assemblages differed relatively little and were all highly comparable to slope and abyssal communities elsewhere in the world in terms of nematode standing stock, diversity and composition on generic level. The upward migration of nematodes as a response to the recently settled phytodetritus was, however, not reflected in the fatty acid patterns of nematodes. Both dual stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and fatty acids (FA), nonetheless, evidenced the coupling between the planktonic food web and deep-sea nematodes at all stations. Gradual shifts in dual stable isotope values and FA compositions of the nematode communities along the Prime Meridian are suggested to relate to variation in the biochemical composition of plankton-derived food sources rather than by changes in community compositions and genus- or species-specific food preferences or metabolic characteristics alone. FA also revealed that bacteria only contribute to a small extent to the diet of deep-sea nematodes and that nematodes may feed year-round on more degraded or constantly available food sources (e.g. fecal pellets, foraminiferans), supplemented by selective feeding on high quality food (phytodetritus) in circumstances of excess fresh labile detritus. The immediate dependency of this dominant benthic taxon in the deep-sea on surface primary production suggests that the deep sea will not remain unaffected from global change phenomena.

Keywords: Southern Ocean · Deep-sea sediments · Meiofauna · Nematoda · Spatial distribution · Fatty acids · Dual stable isotopes · Bentho-pelagic coupling · ANDEEP-SYSTCO

INTRODUCTION

Similar to other ocean basins, benthos in the Southern Ocean (SO) deep sea (i.e. > 1000 m of water depth) is poorly known in comparison with the continental shelf (Clarke and Johnston 2003; Clarke 2008). Moreover, although metazoan meiofauna represent an ubiquitous group of organisms, numerically dominant compared to other metazoans at all water depths (Rex et al. 2006; Wei et al. 2010), mega- and macrofauna are far better documented. Ecological studies on SO metazoan meiofauna so far showed that the communities followed a general

pattern of decrease in abundance with increasing water depth and generally decreasing food availability along the continental shelf and slope (Herman and Dahms 1992; Vanhove et al. 1995; Gutzmann et al. 2004). This corresponds to a pattern found for metazoan meiobenthos in the deep sea worldwide (Mokievskii et al. 2007). As was shown by a large number of studies (reviewed by Heip et al. 1985; Giere 2009), sediment properties and food sources were both of prime importance in explaining the composition of the Antarctic deep-sea meiofauna, particularly nematode communities. Apart from water depth other multiple, interdependent factors such as hydrography, topography, ice coverage, light, temperature, nutrient availability and the structure of the pelagic food web influence the food supply that reaches the seafloor (Grebmeier and Barry 1991; Bathmann et al. 1997; Clarke and Arntz 2006; Arrigo et al. 2008). Because of the occurrence of the Polar Front, seamounts, and a seasonally retreating ice zone, the SO is characterized by a strong latitudinal variation in organic fluxes and zones with a varying degree of seasonality which likely results in spatial and temporal variability in benthic standing stocks.

Mounting evidence on the presence of seasonal detritus depositions in the deep sea came along with contrasting findings on benthic responses to the deposition of phytodetritus (e.g. Billett et al. 1983; Graf 1992; Gooday et al. 1996; Soetaert et al. 1996; Drazen et al. 1998). Although meiofaunal standing stock is generally proportional to food availability (i.e. sediment organic carbon content and chloroplastic pigment equivalents (CPE); e.g. Vanreusel et al. 1995b; Soetaert et al. 2009), the response of metazoan meiofauna such as nematodes is only minimal or absent compared to bacteria, foraminiferans and several macrofaunal taxa to natural and experimentally simulated depositions of phytodetritus (e.g. Gooday 1988; Graf 1992; Pfannkuche 1993; Soltwedel 1997; Galéron et al. 2001; Guilini et al. 2010 and references therein). The only rapid responses found to an episodic food supply in the deep sea were an increase in mean nematode size at the German BIOTRANS site (April - July; Soltwedel et al. 1996) and nematode migration towards the sediment surface in the Polar Front (December - January; Veit-Köhler et al. 2011). Doubled meiofaunal abundance (mainly nematodes), from summer to autumn in sediments from the bathyal Mediterranean (de Bovée et al. 1990) and the Northeast Atlantic along the Hebridean margin (Mitchell et al. 1997) indicate, however, a response on a longer time scale. Therefore, till today, it is assumed that deep-sea nematodes are feeding additionally or alternatively on rather unlimited, other food sources than phytodetritus (e.g. bacteria, ciliates, flagellates, foraminiferans, fungi, DOC) and/or are characterized by slower respiration or somatic growth rates (e.g. Giere 2009). Rigid evidence on any of these assumptions is, however, lacking. While gut content analysis is a technique that is not applicable due to size restrictions of deep-sea nematodes, the use of natural stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) and fatty acid compositions (FA) provides an alternative to obtain a more time-integrated view on the diet of organisms. Although both techniques are

widely spread in marine ecological studies, the latter technique was only applied twice on marine free-living nematodes (Leduc 2009; Van Gaever et al. 2009c).

ANDEEP I to III (ANtarctic DEEP-sea benthic diversity: colonisation history and recent community patterns) were pioneer projects in the study of the Antarctic deep-sea benthos. By sampling mega-, macro- and meiofauna of no more than 40 stations in the Atlantic sector of the SO, ANDEEP revealed the scale and patterns of unknown species diversity and gained insight in the potential origin of the abyssal SO fauna, its degree of endemism as well as bathymetric ranges of species (Brandt and Hilbig 2004; Brandt et al. 2007a, b). ANDEEP-SYSTCO (ANDEEP-SYSTem COupling) started in the austral summer 2007/08 and builds on the precursor programme ANDEEP, with as main goal to understand the processes that shape the abyssal communities. Therefore SYSTCO aims to investigate functional biodiversity and ecology of abyssal communities of the Atlantic sector of the SO, focusing on the role of key organisms, which frequently occur in samples, including their general feeding biology (Brandt and Ebbe 2009). Since the benthic fauna depends on deep carbon export from pelagic production, a station located in the Polar Front (52°2′ S, 0°1′ W) where a phytoplankton bloom had settled during the course of the expedition, was revisited after 52 days to perform repeated measurements and elucidate the coupling processes between the ocean surface and deep-sea floor.

This study evaluates the spatial distribution of deep-sea nematode assemblages and their relationships with environmental conditions (water depth, grain size distribution, nitrogen and carbon content and chl-*a* pigment concentration) along a north-south transect following the Prime Meridian across the SO (49° S - 70° S). Nematode assemblages are also considered with regards to the short-term response on the seasonal deposition of particulate organic matter (POM) within the SO Polar Front. Nematode fatty acid composition and natural stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) are determined to provide a time-integrated view on the organisms' diets on community level. Stable isotope signals of aquatic animals typically increase ca. 0.5‰ in $\delta^{13}C$ and 2.3‰ to 3.4‰ in $\delta^{15}N$ per trophic step (Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003), while the FA marker concept relies on the fact that certain FA are incorporated into consumers in a conservative manner (e.g. Dalsgaard et al. 2003; Lee et al. 2006). By comparing both nematode fatty acid and dual stable isotope signals with what is known from literature on surface water POM, other benthic organisms, bulk sediment and bottom water POM properties, insight is gained in the resources used by deep-sea nematode communities and the degree of bentho-pelagic coupling across the Southern Ocean.

Overall, the following hypotheses were tested:

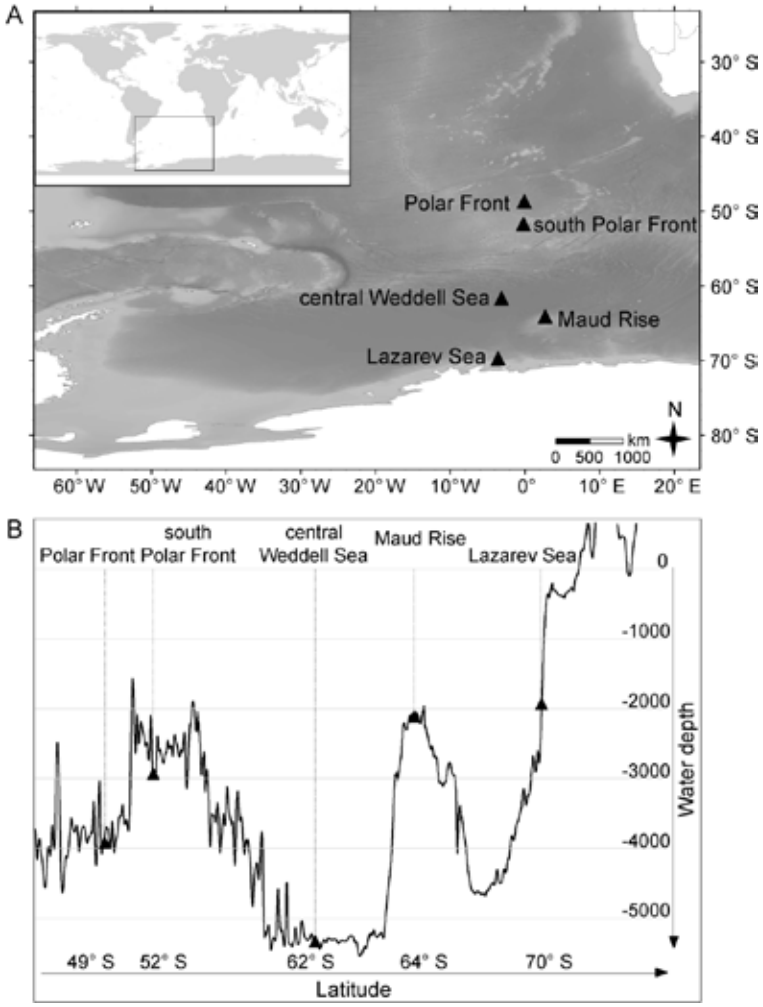
1. Nematode community structure and trophic ecology do not differ along a latitudinal gradient across the deep SO
2. Deep-sea nematodes at the Polar Front do not respond rapidly to a seasonal pulse of organic matter to the seafloor

MATERIAL AND METHODS

Study site and sampling procedure

Samples were collected during the ANDEEP-SYSTCO expedition on board of the RV *Polarstern* (ANT XXIV/2, 28.11.2007 - 04.02.2008). Sediment samples were taken at depths from 1935 to 5323 m, at six sites oriented north-south in close proximity of the Prime Meridian between 49° S and 70° S; across the Polar Front, the Weddell Sea and the Lazarev Sea (Fig. 1, Table 1). The stations are further indicated with their abbreviations (PF: Polar Front, sPF: south Polar Front, cWS: central Weddell Sea, MR: Maud Rise, LS: Lazarev Sea). All stations occur in different water masses and environments with different topographical and sedimentary conditions. The PF, sPF and cWS station are situated on the abyssal sea floor, MR is a seamount and LS is located on continental slope. The station located at a southern position in the Polar Front (sPF, at 52° S) was visited twice; once when a phytoplankton bloom was detected in the euphotic water layer based on fluorescence (06.12.2007; Herrmann and Bathmann 2010) and 52 days later when the remains of the bloom had settled to the sea floor (26 - 27.01.2008). These remains were visible as a greenish fluff layer on the sediment surface (Veit-Köhler et al. 2011). Further details on sampled stations are available in the cruise report (Bathmann 2010).

Figure 1. Location of the ANDEEP-SYSTCO stations. A. Bathymetric map situating the stations across the Southern Ocean, along the Prime Meridian. Exact coordinates are given in Table 1. B. Cross-section of the bathymetry along the transect with indication of the stations.



All meiobenthic samples were taken with a multiple corer (MUC) sampling device equipped with 12 plexiglass cores (inner diameter: 9.4 cm, equivalent to 96.4 cm²). The MUC was deployed successfully one to three times per station, depending on ship time availability and sea state. The processed samples were thus a combination of true replicates and pseudo-replicates, since different cores from the same MUC deployment do not meet the criteria of random sampling (Hurlbert, 1984). An overview of the processed samples is given in Table 1. The sediment cores destined for meiofauna community analysis were sliced into

one-centimeter fractions down to five centimeters and together with the supernatant water preserved in a borax-buffered 4% formaldehyde-seawater solution. From one core per deployment, syringe subsamples (2 cm diameter) were taken for granulometry. The syringes were also sliced into one-centimeter fractions down to five centimeters and stored at -20 °C. Furthermore a minimum of four cores per deployment were used to scoop off the upper five centimeters of sediment. This material was immediately sieved in the lab with filtered seawater (32 µm mesh) over stacked sieves (1 mm, 500 µm, 100 µm and 32 µm) to prevent clogging. We retained the 500 µm, 100 µm and 32 µm fractions and stored them at -80 °C. Back at the laboratory, the nematodes were extracted for biochemical analyses (fatty acids and stable isotopes) after the different size fractions were pooled again.

Sediment parameters

Grain size analyses on the one centimeter sediment slices were carried out using a Coulter Counter LS 100TM Particle Size Analyser. The 0.06 - 1000 µm sediment fractions were expressed in volume percentages (vol %) and classified according to Wentworth (1922). Detailed results on the sediment grain size characteristics per station are published in Brenke et al. (2011). Total sedimentary nitrogen and organic carbon were determined on freeze-dried, homogenised, acidified (with 1% HCl) and dried one centimeter sediment slices using a Flash EA 1112 + Mas 200 elemental analyser (Thermo Interscience; combusted at 1000 °C). Sediment-bound chlorophyll-*a* pigment (chl-*a*) concentrations per sediment centimeter slice were determined with a fluorescence detector after extraction in acetone and separation using reversed phase high-performance liquid chromatography (HPLC) on lyophilised and homogenised sediment samples according to Wright and Jeffrey (1997) or Hoffmann et al. (2006), depending on the laboratory where the analysis was performed.

Nematode parameters

Sediment samples destined for identifying the nematode community were rinsed with tap water over a 32-µm mesh sieve (no upper sieve size used). The fraction retained on the 32-µm sieve was three times centrifugated with the colloidal silica polymer Levasil® (H.C. Stark, 200/40%, ϱ = 1.17, for 6 minutes at 4000 rpm) and kaolin (McIntyre and Warwick 1984) to extract all organisms. After staining with Rose Bengal, all metazoan organisms were manually sorted to a higher taxon level (following Higgins and Thiel 1988) and counted under a Leica MZ 12.5 stereomicroscope (8 - 100x magnification). Where possible, about 50 to 100 nematodes were picked out randomly with a fine needle from each centimeter sediment layer. They were gradually transferred to glycerine (Seinhorst 1959) before being mounted on glass slides. Nematodes were identified to genus level under a compound microscope (1000×

magnification). Adults were distinguished from juveniles based on the development of a vulva and uterus in females and a gonad and spicules in males. Based on mouth morphology, all identified individuals were classified into four feeding type groups according to Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth or epistratum feeders (2A), and predators/scavengers (2B). By using a Leica DMR compound microscope and Leica LAS 3.3 imaging software, nematode length (L, filiform tail excluded) and maximal width (W) were measured. Nematode biomass was then calculated with Andrassy's formula (Andrassy 1956): wet weight (µg) = L (µm) x W^2 (µm) / 1.6 10^6, and a dry-to-wet-weight ratio of 0.25 was assumed (Heip et al. 1985). To calculate total biomass of each sediment layer and sampling station, total nematode densities were taken into account.

Samples that were stored frozen and destined for stable isotope and fatty acid analysis, were thawed and triple centrifugated with Levasil® and kaolin (6 minutes at 4000 rpm) to extract the meiofauna. The extracted meiofauna was rinsed with MilliQ water and processed immediately. These samples served two purposes. On the one hand, minimum 900 nematode individuals per station were handpicked with a fine sterile needle and rinsed in MilliQ water to remove adhering particles. Subsequently they were transferred to a drop of MilliQ water in 3.5 x 5.0 mm tin cups. These cups were oven-dried overnight at 30 °C, pinched closed and stored in Microtitre plates under dry atmospheric conditions (40 °C) until dual $\delta^{13}C$ and $\delta^{15}N$ analysis. An isotope ratio mass spectrometer (Delta Plus, Thermo-Finnigan) coupled to an elemental analyzer (NC 2500, Carlo Erba; combustion temperature 1080 °C) at the GeoBio-Center (University of Munich, Germany) was used to measure dual carbon and nitrogen stable isotope ratios and elemental contents (wt% N and C) on the same sample. Stable isotope ratios are expressed in the δ notation with Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 (AIR) as reference standards for carbon and nitrogen, respectively. Both stable isotope signatures are expressed in units of ‰, according to the conventional formula $\delta^{13}C$ (or $\delta^{15}N$) = $[R_{sample} / R_{standard} - 1] \times 10^3$, where R is the ratio $^{13}C/^{12}C$ (or $^{15}N/^{14}N$) of the sample or the aforementioned standards. Carbon and nitrogen contents were determined from the peak area versus sample weight ratio of each sample using cyclohexanone-2,4-dinitrophenylhydrazone ($C_{12}H_{14}N_4O_4$) and atropine ($C_{17}H_{23}NO_3$, both Thermo Quest) for calibration. A lab standard (peptone) calibrated against IAEA standards was used as isotope standard. Samples outside the linearity range were corrected using peptone standards with equivalent peak areas. Analytical precision (one standard deviation) was 0.2‰ and 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively, calculated from peptone standards with variable weights in the range of the samples and from the respective series of measurements.

On the other hand the fatty acid (FA) composition of nematodes in bulk was determined. Therefore, two to three times 300 to 550 bulk nematode individuals per station (equalling 26 to 54 µg C) were handpicked with a fine sterile needle. After rinsing in MilliQ water to

remove adhering particles they were transferred in MilliQ water into 4.0 ml GC vials with a minimum of MilliQ water and frozen again at -80 °C before freeze-drying. Hydrolysis of total lipids and methylation to fatty acid methyl esters (FAME) for FA analysis was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008). The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4-methanol solution since BF_3-methanol can cause artefacts or loss of polyunsaturated FA (PUFA; Eder 1995). The fatty acid methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification. Samples were centrifuged (Eppendorf centrifuge 5810R) and vacuum dried (Rapid Vap LABCONCO). The FAME obtained from the replicate extracts of the nematodes in each of the 5 locations were analysed using a gas chromatograph (Hewlett Packard 6890N) coupled to a mass spectrometer (HP 5973). All samples were run in splitless mode, with a 5 µL injection per run, at an injector temperature of 250 °C, using a HP88 column (60m × 25mm internal diameter, Df = 0.20; Agilent J&W; Agilent Co., USA) with He flow rate of 1.3 ml min^{-1}. The oven temperature was programmed at 50 °C for 2 minutes, followed by a ramp at 25 °C min^{-1} to 75 °C, then a second ramp at 2 °C min^{-1} to 230 °C with a final 4 minutes hold. FAME were identified by comparison with the retention times and mass spectra of authentic standards and available ion spectra in WILEY mass spectral libraries, and analysed with the software MSD ChemStation (Agilent Technologies). Quantification of individual FAME was accomplished by the use of external standards (SupelcoTM 37 Component FAME Mix, Supelco # 47885, Sigma-Aldrich Inc., USA). The quantification function of each individual FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 150 µg mL^{-1}). Shorthand FA notations of the form A:BωX were used, where A represents the number of carbon atoms, B gives the number of double bonds and X is the position of the double bond closest to the terminal methyl group (Guckert et al. 1985). Some FA used as biomarkers for the type of ingested food are of special interest and widely used for the interpretation of an organism's FA composition. Therefore we grouped the planktonic markers 16:1ω7, 18:1ω9, 18:2ω6, 20:PUFA, 22:PUFA; and the bacterial markers 15:0, 17:0, 15:1, 17:1, *iso* and *anteiso*-branched SFA and MUFA, 10-methylpalmitic acid, *trans*-16:1ω7, 18:1ω7c, cy17:0 and cy19:0 (e.g. Dalsgaard et al. 2003 and references therein; Camacho-Ibar et al. 2003 and references therein; Desvilettes et al. 1997; Gurr and Harwood 1991).

Statistical analysis

Statistical analyses were conducted using PRIMER version 6 with PERMANOVA+ add-on software (Clarke and Gorley 2006; Anderson et al. 2008). Prior to the analyses, nematode density and biomass data of the upper five centimeter layers were summed taking into account the individual counts per depth layer. Structural diversity Hill's indices that are

variably dependent on relative genera abundances (H_0 = number of genera, H_1, H_2, H_{inf}; Hill 1973) and a rarefaction index, i.e. the expected number of genera present in a population of 100 individuals (EG(100); Hurlbert 1971), were generated based on these density data. To assess functional diversity, the trophic diversity index Θ^{-1} was calculated (Heip et al. 1988). Along the transect both univariate and multivariate non-parametric permutational ANOVAs (PERMANOVA) were performed to test for differences in nematode density, biomass, diversity, $\delta^{13}C$ and $\delta^{15}N$ values, FA content and nematode genus and FA composition, respectively. One replicate fatty acid sample (cWS) was omitted from the dataset due to exorbitant high measured concentrations compared to the other samples. Euclidean distance similarity was used as resemblance measure on untransformed univariate data, while Bray-Curtis similarity was used as resemblance measure on standardized and square root or log (x+1) transformed genus abundance and FA data, respectively. Both models had an unbalanced one factor design including the fixed categorical factor 'location'. Calculation of the Pseudo-F ratio and p value required unrestricted permutation of raw data (univariate) or 9999 permutations of the residuals under a reduced model (multivariate). The results of posteriori pair-wise tests were, however, only based PERMDISP confirmation of homogeneity of multivariate sample dispersions in case where the different centimeter layers were included (sPF versus sPF (2^{nd} visit) stations) since other compared groups consisted of less than 3 samples. Nevertheless, pairwise tests clarified between which locations or sediment depth layers that the significant differences identified by the main tests were found. Because of the restricted number of unique permutations in the pairwise tests, p values were obtained from Monte Carlo samplings (Anderson and Robinson 2003). A non-metric multidimensional scaling (MDS) plot was constructed to visualize the nematode community composition among all stations. To visualize the differences in nematode FA compositions among the stations a Principal Coordinates analysis (PCO) was used. PCO is comparable to MDS in that it is very flexible - it can be based on any (symmetric) resemblance matrix. However, it is also like a PCA, in that it is a projection of the points onto axes that minimize residual variation in the space of the resemblance measure chosen. Due to the limited sample-to-variable ratio, variables represented by less than five samples were deleted to improve the reliability and reproducibility of the analysis as proposed by Tabachnick and Fidell (1989) for PCA analyses (Budge et al. 2006). In addition, a new feature provided as part of the PERMANOVA+ add-on is the ability to superimpose vectors onto the plot that correspond to the raw correlations of individual variables with the ordination axes. We chose the Spearman rank correlation that shows monotonic increasing or decreasing relationships with axes. The vector overlay includes only those FA that correlated with more than 60% with one of the first two PCO-axes. Additional DISTLM analyses identified the percentage of variability in nematode community and FA compositions between stations that was explained by the available environmental factors. The *Adjusted* R^2 criterion and *Step-wise* selection procedure were

chosen to select the best possible combination of predictor variables available.

To test the effect of a settled phytoplankton bloom on different nematode parameters (community composition, total relative abundance, total and mean individual biomass, juvenile versus adult ratio) over 5 cm depth at the sPF station, multivariate and univariate PERMANOVA analyses with a balanced fully crossed three factor design were used. The model included the fixed categorical factors ‘time’ and ‘sediment depth’ with the random ‘replicate’ factor nested in ‘time’ (since data from different depth layers from a single replicate core are not fully independent), and all interaction terms. Both multivariate and univariate analysis were run as described above.

Table 1. Overview of the ANDEEP-SYSTCO stations, deployments per station and core codes of the samples that were processed for meiofaunal densities and additionally for nematode community composition, diversity and biomass (in bold). Date, depth, longitude and latitude are listed. Sediment environmental parameters (mean grain size, sediment fraction volume percentages, nitrogen and organic carbon content, chl-a) were integrated over 5 cm. Per station, the labile portion of the organic carbon flux (LC_{org}), as calculated by Sachs et al. (2009) based on in situ and ex situ measurements of O_2 profiles in surface sediments, is shown.

Location	Station-Deployment (Core Code)	Date	Depth (m)	Latitude	Longitude
PF	090-2 (**2** - **8**)	29.01.2008	3980	49° 0.95′ S	0° 0.03′ E
sPF	013-12 (2 - **6** - **12**)	06.12.2007	2963	52° 2.22′ S	0° 1.04′ W
sPF	013-14 (1 - **4** - 9)	06.12.2007	2970	52° 2.25′ S	0° 1.11′ W
sPF (2nd visit)	085-5 (2 - **3** - 11)	26.01.2008	2965	52° 1.20′ S	0° 0.20′ E
sPF (2nd visit)	085-7 (**1** - **4** - 8)	27.01.2008	2964	52° 1.53′ S	0° 0.16′ E
cWS	033-10 (**3** - **4**)	30.12.2007	5323	62° 0.80′ S	2° 59.05′ W
MR	039-10 (**5**)	03.01.2008	2116	64° 28.83′ S	2° 52.48′ E
MR	039-12 (**8**)	03.01.2008	2123	64° 28.83′ S	2° 52.53′ E
MR	039-14 (**11**)	03.01.2008	2119	64° 28.84′ S	2° 52.49′ E
LS	017-12 (**6** - 8 - 11)	22.12.2007	1935	70° 4.86′ S	3° 22.59′ W
LS	017-14 (**1** - 11 - **12**)	22.12.2007	1951	70° 4.80′ S	3° 22.71′ W

Mean Grainsize (µm)	Clay (Vol %)	Silt (Vol %)	Very Fine Sand (Vol %)	Coarse Sand (Vol %)	Nitrogen content (%)	Carbon content (%)	Chl-*a* (ng/g dry sediment)	LC_{org} flux (mg C m^{-2} d^{-1})
32.27	17.90	71.62	7.92	2.55	0.03	0.45	12.02	2.4
36.37	10.85	77.23	9.93	1.98	-	-	-	
42.80	9.35	75.94	10.11	4.59	-	-	28.14	3.3
31.68	10.26	79.00	10.12	0.61	-	-	61.08	
34.22	10.85	77.46	10.00	1.69	-	-	-	8.4 ± 1.0
12.00	33.12	65.04	1.37	0.47	0.08	0.47	3.67	3.0
44.70	10.73	71.21	11.11	6.95	0.04	0.30	312.23	
48.07	11.17	69.01	11.59	8.23	0.09	0.56	0.00	2.1 ± 0.4
44.77	11.19	70.41	11.42	6.98	0.05	0.29	0.00	
58.70	16.03	55.97	16.18	11.82	0.03	0.18	-	
57.95	18.26	56.64	14.16	10.71	0.02	0.17	7.02	2.0

Table 2. Nematode relative abundance, density, biomass, diversity (H_0, H_1, H_2, H_{inf}, $E(G_{100})$) and relative abundance of the nematode feeding types and trophic diversity (Θ^{-1}) for the top 5 cm of the sediment, averaged per location (± standard deviation).

Location	Nematode relative abundance (%)	Nematode density (ind. 10 cm^{-2})	Nematode biomass (µg dwt 10 cm^{-2})	H_0	H_1	H_2
PF	90.9 ± 0.1	69 ± 5	11.3 ± 2.6	49.5 ± 4.9	24.7 ± 2.5	14.2 ± 0.4
sPF	85.5 ± 2.6	693 ± 258	105.7 ± 11.5	55.3 ± 2.9	26.5 ± 1.0	17.5 ± 2.0
sPF (2nd visit)	80.5 ± 3.3	480 ± 48	62.5 ± 21	54.3 ± 3.1	26.5 ± 1.4	17.3 ± 0.9
cWS	92.3 ± 2.8	648 ± 145	159.0 ± 58.3	47.5 ± 0.7	18.8 ± 1.1	10.4 ± 0.2
MR	92.6 ± 0.3	1102 ± 233	324.1 ± 175.5	45.3 ± 3.2	24.2 ± 3.7	17.2 ± 3.3
LS	86.4 ± 2.5	833 ± 132	294.5 ± 145.7	57.0 ± 1.0	30.8 ± 3.0	21.7 ± 3.8

Table 3. Results from main and pairwise one-factor multivariate and univariate PERMANOVA analyses testing for differences in nematode community composition, nematode fatty acid composition and nematode density, biomass, structural diversity and functional diversity, and nematode $\delta^{13}C$ and $\delta^{15}N$ values, respectively, in the top five centimeters of the sediment among all locations. Significant differences are indicated in bold. ([a]: $0.001 < p \leq 0.05$; [b]: $p \leq 0.001$)

Factor		Nematode density	Nematode biomass	Nematode community			
					H_0	H_1	H_2
Location	df	5	5	5	5	5	5
	MS	1.6 10^7	43076	1758	62.2	37.4	34.2
	Pseudo-F	**10.99** [b]	**3.96** [a]	**3.33** [b]	**7.49** [a]	**6.41** [a]	**5.67** [a]
Residuals	df	19	10	10	10	10	10
	MS	1.4 10^6	10865	528.6	8.3	5.8	6.0
Total	df	24	15	15	15	15	15
PF, sPF	t	**3.40** [a]	**10.86** [a]	1.86	1.73	1.20	2.22
PF, sPF (2nd visit)	t	**11.90** [b]	**3.17** [a]	1.85	1.40	1.04	**4.45** [a]
PF, cWS	t	**5.63** [a]	3.58	1.85	0.57	3.06	**12.56** [a]
PF, MR	t	**5.96** [a]	2.39	**2.19** [a]	1.18	0.17	1.25
PF, LS	t	**7.60** [b]	2.61	1.81	2.76	2.35	2.65
sPF, sPF (2nd visit)	t	1.67	**3.06** [a]	1.03	0.41	0.03	0.14
sPF, cWS	t	0.08	1.67	1.66	**3.59** [a]	**8.42** [a]	**4.75** [a]
sPF, MR	t	**2.72** [a]	2.15	1.78	**4.01** [a]	1.06	0.12
sPF , LS	t	1.57	2.24	1.53	0.94	2.38	1.71
sPF (2nd visit), cWS	t	**2.59** [a]	2.78	**2.02** [a]	2.96	**6.34** [a]	**9.97** [a]
sPF (2nd visit), MR	t	**6.10** [b]	2.56	**1.91** [a]	**3.52** [a]	1.01	0.05
sPF (2nd visit), LS	t	**5.52** [b]	2.73	**1.80** [a]	1.44	2.27	1.95
cWS, MR	t	2.40	1.23	**2.33** [a]	0.89	1.93	2.79
cWS, LS	t	1.66	1.20	1.75	**11.4** [a]	**5.22** [a]	**3.96** [a]
MR, LS	t	2.26	0.58	**2.13** [a]	**6.00** [a]	2.43	1.56

H_{inf}	EG(100)	Feeding types				Θ^{-1}
		1A	1B	2A	2B	
5.7 ± 0.0	35.8 ± 2.5	53 ± 2	16 ± 1	24 ± 4	8 ± 1	2.7 ± 0.1
7.8 ± 1.4	34.6 ± 1.0	30 ± 5	22 ± 1	42 ± 4	6 ± 2	3.1 ± 0.1
7.4 ± 0.8	33.5 ± 2.5	30 ± 7	15 ± 1	51 ± 6	4 ± 1	2.6 ± 0.1
4.2 ± 0.1	28.8 ± 0.6	28 ± 2	43 ± 4	26 ± 0	3 ± 2	3.0 ± 0.2
7.2 ± 0.9	33.7 ± 2.4	24 ± 2	17 ± 1	51 ± 4	9 ± 3	2.9 ± 0.2
9.5 ± 2.7	39.0 ± 1.0	29 ± 6	28 ± 6	33 ± 4	11 ± 2	3.5 ± 0.1

Nematode diversity			Nematode $\delta^{13}C$	Nematode $\delta^{15}N$	Nematode fatty acids
H_{inf}	EG(100)	Θ^{-1}			
5	5	5	5	5	5
7.8	26.7	0.3	24.3	5.76	253.1
3.71 [a]	**7.75** [a]	**12.67** [a]	**91.42** [b]	**16.10** [b]	**4.31** [b]
10	10	10	20	19	13
2.1	3.4	0.02	0.27	0.36	58.7
15	15	15	25	24	18
2.07	0.78	**4.04** [b]	0.65	**4.85** [a]	1.75
2.98	1.00	0.57	1.24	1.74	2.15
20.4 [a]	3.86	2.37	**16.80** [a]	**854.40** [b]	1.19
2.21	0.95	1.16	**17.43** [b]	**5.79** [a]	**2.34** [a]
1.85	2.15	**10.33** [a]	**6.46** [b]	**4.04** [a]	**3.71** [a]
0.48	0.72	4.54 b	1.92	1.67	1.67
3.56 [a]	**7.14** [a]	0.93	**5.03** [a]	**5.07** [a]	1.20
0.73	0.63	1.49	**13.66** [b]	**4.94** [a]	1.57
0.92	**5.40** [a]	**4.04** [b]	**5.75** [b]	0.84	**2.60** [a]
5.67 [a]	2.46	2.77	**11.40** [b]	**3.90** [a]	1.56
0.37	0.09	1.72	**23.22** [b]	**6.11** [b]	**1.88** [a]
1.26	**3.56** [a]	**9.42** [a]	**10.26** [b]	**2.48** [a]	**3.36** [a]
4.55 [a]	2.66	0.54	**5.67** [a]	0.50	1.13
2.61	**12.61** [a]	**4.49** [b]	1.14	**2.99** [a]	**2.75** [a]
1.40	**3.55** [a]	**4.26** [b]	**8.16** [b]	**4.43** [b]	**2.48** [a]

RESULTS

Community structure along the transect

Nematodes dominated the metazoan meiofauna at all locations, with a dominance ranging from 80.5 ± 3.3% to 92.6 ± 0.3% (Table 2). Nematode densities ranged from 69 ± 5 ind. 10 cm^{-2} to 1102 ± 233 ind. 10 cm^{-2}, with lowest densities found at the PF station and highest at the MR station. PERMANOVA found significantly lower nematode densities between PF and sPF (2nd visit) compared to all other stations (Table 3). Nematode biomass ranged from 11.3 ± 2.6 µg dwt 10 cm^{-2} at the PF station to 324.1 ± 175.5 µg dwt 10 cm^{-2} at the MR station. Significant differences were only found between the PF and the sPF (both pre- and post-bloom settlement) stations, and between both sampling times at the sPF station (Table 3).

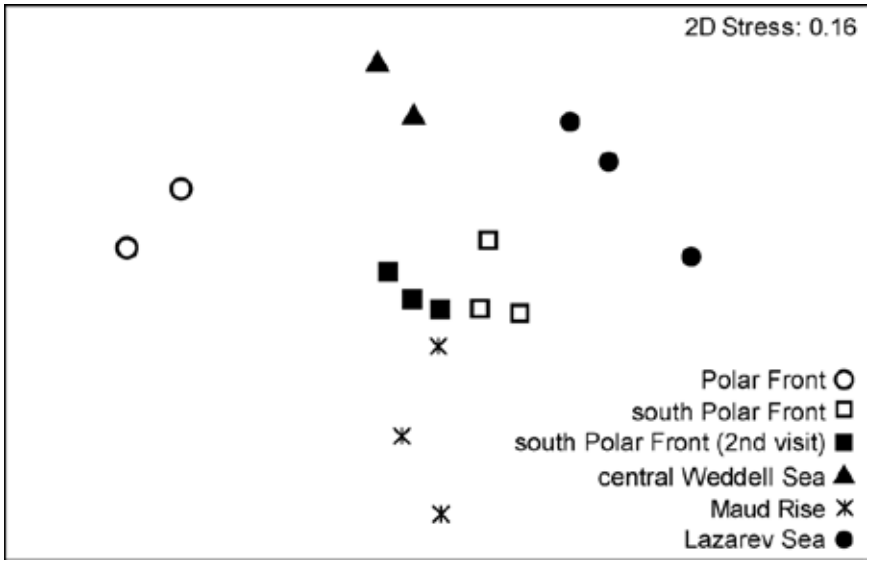
A total of 137 genera belonging to 36 families were identified. An overview of the dominant genera per station, with a relative abundance higher than 2%, is given in Table 4. The four genera that dominate at all stations, though with varying abundances, are *Desmoscolex* (16.8% - 3.3%), *Acantholaimu*s (13.5% - 6.2%), *Tricoma* (6.8% - 2.3%) and *Halalaimus* (7.9% - 2.8%). Only between 6.0% and 16.6% of the genera were present with abundances lower than 1%. The percentage of genera that occurred exclusively at one station ranged from 2.1% at the MR station to 9.0% at the LS station. These exclusive genera only accounted for 0.4% to 2.7% of the total abundances. At the sPF station *Acantholaimus* was the most dominant genus before (13.0%) as well as after (12.4%) the bloom settled. All other dominant genera that occurred at the pre-bloom situation remained dominant over time, although with small variations in relative abundances. Additionally, *Leptolaimus* only appeared after the bloom settled (3.0%). PERMANOVA results on the standardized and square root transformed multivariate data set showed that the nematode assemblages differed among the stations (Table 3). Pairwise comparisons revealed that significant differences were only found between the station MR and all other stations, except for the sPF station; and between the stations sPF (2nd visit) and cWS and LS (Table 3). No significant difference was found before and after the bloom had settled to the seafloor. These results were visualised in an MDS plot (Fig. 2). Environmental variables that were relevant in explaining the variation in nematode assemblages were the volume percentage of clay (14.8%, $p = 0.003$) and very fine sand (14.0%, $p = 0.005$), and water depth (13.4%, $p = 0.001$).

Diversity indices differed significantly among the stations (Table 3). Since the differences are index dependent, and no consistency occurred, the averages are interpreted. Average structural diversity indices were all highest for the LS station (Table 2), regardless of the emphasis given to relatively more or less abundant genera. Besides the highest average number of genera ($H_0 = 57 \pm 1$) and expected number of genera on 100 individuals (EG(100)

= 39 ± 1), genera also occurred most evenly (H_{inf} = 9.5 ± 2.7), with *Acantholaimus* being the most dominant genus (9.2%). While the lowest number of genera was found at the MR station (45.3 ± 3.2), Hill's diversity indices indicated lowest evenness due to highest dominance of one genus (*Theristus*: 23.9%) at the deepest station, cWS. In terms of evenness, diversity more or less decreased with water depth.

Based on mouth morphology, nematode assemblages were dominated by epistratum (or epigrowth) feeders at most stations (sPF: 42 ± 4%, sPF (2nd visit): 51 ± 6%, MR: 51 ± 4% and LS: 33 ± 4%), followed by selective deposit (24 ± 2% - 30 ± 5%) and non-selective deposit feeders (15 ± 1% - 28 ± 6%). At the PF station the proportion of epistratum (24 ± 4%) and selective deposit feeders (53 ± 2%) was reversed, while at the cWS station non-selective deposit feeders dominated (43 ± 4%), followed by epistratum (28 ± 2%) and selective deposit feeders (26%) (Table 2). Predators and scavengers were least present at all stations (3 ± 2% - 11 ± 2%). Trophic diversity ranged from 2.6 ± 0.1 at the sPF (2nd visit) station to 3.5 ± 0.1 at the LS station (Table 2), with a significantly higher trophic diversity at the LS station compared to all other stations (Table 3).

Figure 2. Non-parametric multi-dimensional scaling (MDS) plot of the nematode communities in the top five centimeters of the sediment along the ANDEEP-SYSTCO transect.



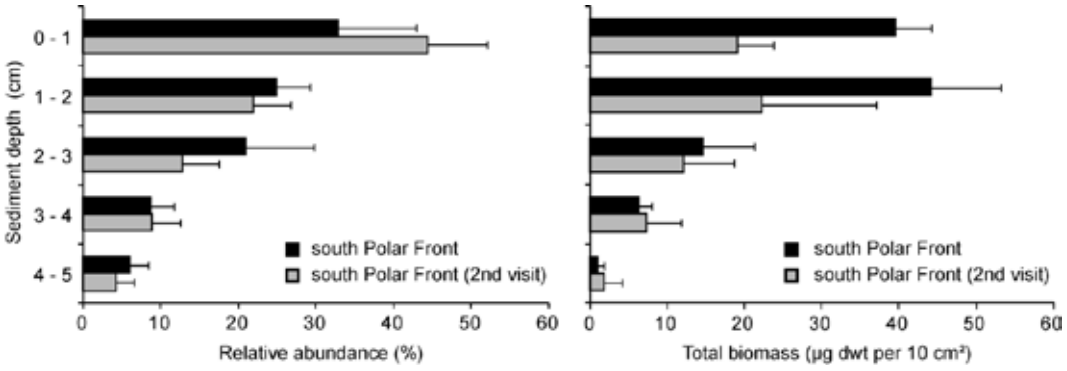
PF	%	sPF	%	sPF (2nd visit)	%	cWS	%	MR	%	LS	%
Desmoscolex	16.8	*Acantholaimus*	13.0	*Acantholaimus*	12.4	*Theristus*	23.9	*Actinonema*	11.7	*Acantholaimus*	9.2
Acantholaimus	13.5	*Daptonema*	8.1	*Microlaimus*	10.6	*Acantholaimus*	11.5	*Desmodora*	11.4	*Sabatieria*	8.8
Tricoma	6.8	*Halalaimus*	7.9	*Chromadorina*	8.0	*Desmoscolex*	7.8	*Microlaimus*	6.6	*Desmoscolex*	6.9
Monhystrella	6.1	*Actinonema*	6.3	*Halalaimus*	6.0	*Daptonema*	7.8	*Acantholaimus*	6.2	*Dichromadora*	5.1
Halalaimus	4.0	*Desmoscolex*	5.6	*Actinonema*	4.6	*Halalaimus*	4.5	*Chromadorina*	5.9	*Cervonema*	4.7
Gammanema	3.5	*Desmodorella*	4.4	*Tricoma*	4.6	*Dichromadora*	4.2	*Paramesacanthion*	5.4	*Daptonema*	4.7
Leptolaimus	3.5	*Monhystrella*	4.3	*Monhystrella*	4.5	*Tricoma*	4.1	*Enchonema*	3.8	*Chromadorina*	3.5
Prototricoma	3.3	*Chromadorina*	4.0	*Desmoscolex*	4.1	*Monhystrella*	3.3	*Dichromadora*	3.5	*Halalaimus*	3.3
Diplopeltoides	2.9	*Tricoma*	3.5	*Daptonema*	3.7	*Intasia*	3.0	*Leptolaimus*	3.5	*Microlaimus*	3.3
Procamacolaimus	2.2	*Pselionema*	2.9	*Desmodora*	3.3	*Microlaimus*	2.7	*Thalassomonhystera*	3.4	*Intasia*	2.9
		Microlaimus	2.9	*Leptolaimus*	3.0	*Enchonema*	2.2	*Desmoscolex*	3.3	*Bolbolaimus*	2.8
		Intasia	2.5	*Intasia*	3.0	*Prototricoma*	2.2	*Halalaimus*	2.8	*Desmodorella*	2.6
		Paracantonchus	2.5	*Paracantonchus*	2.9	*Leptolaimus*	2.1	*Tricoma*	2.5	*Tricoma*	2.3
		Desmodora	2.2	*Pselionema*	2.7			*Pselionema*	2.1	*Actinonema*	2.1
				Desmodorella	2.4					*Theristus*	2.0
Rest	37.5	Rest	29.8	Rest	24.2	Rest	20.5	Rest	27.9	Rest	35.7

Table 4. Mean relative abundance of dominant genera (> 2%) per station, over 5 cm sediment depth.

Temporal variability at the south Polar Front

Between pre and post settlement of the bloom, the nematode community composition did not change (Table 3). This was also the case where PERMANOVA considered each centimeter separately (Appendix A). Although total nematode densities per centimeter layer did not change before and after the bloom settled (Appendix A), a significantly higher portion of nematodes was found in the top centimeter after compared to before the bloom settled (44.5 ± 7.5% vs. 32.8 ± 10%, respectively; Appendix A). A significant difference in total nematode biomass was found based on the interaction term 'Time x Sediment depth' (Appendix A). Nevertheless, a PERMDISP analysis revealed that this difference was due to a difference in relative data dispersion between the two sampling times. Therefore no valid pairwise comparison between the different sediment depth layers of the different sampling times could be performed. Neither did other nematode variables such as mean individual biomass and relative proportions of juveniles and adults (PF: 28.6 ± 4.7% juveniles, 71.4 ± 4.7% adults; PF(2nd visit): 27.7 ± 5.7% juveniles, 72.3 ± 5.7% adults) change over time (Appendix A).

Figure 3. Nematode relative abundance (%) and total biomass (µg dwt per 10 cm²) per centimeter from the south Polar Front station, before (black bars) and after (grey bars) the bloom settled. The averages and standard deviation of triplicates are plotted.



Fatty acid compositions

At all stations, PUFA dominated in nematodes (34.2% to 45.3 ± 9.5%; Table 5). When FA markers were allocated to the potential food source, planktonic FA (Σ 16:1ω7c, 18:1ω9, 18:2ω6, 20:PUFA, 22:PUFA) were dominant in nematodes at all stations, with values varying from 59.4 ± 3.0% at the sPF station to 67.5 ± 3.7% at the PF station. Only a few bacterial FA biomarkers were found in the nematodes (15:0, 17:0, 17:1ω7c). Together they accounted

for a maximum of 5.7 ± 0.9% of the total amount of FA at the PF station. The predominant individual fatty acids that reached more than 10% relative abundance at one of the stations were 14:0, 16:0, 18:1ω9t, 18:1ω9c, 20:4ω6, 20:5ω3, and 22:6ω3. Total FA content varied from 0.01% at the PF station to 3.6 ± 2.4% at the cWS station of bulk dry weight, with no significant differences between stations (Main test, P(perm) = 0.069). The PERMANOVA analysis indicated a difference in FA composition between the LS station and all other stations, and between the MR station and the PF and sPF (2nd visit) stations (Table 3). PCO explained 54.5% of total variance in the first two axes (29% and 25.5%, respectively) (Fig. 4). The vector overlay indicates FA-specific correlating of more than 60% with one of the first two PCO axes, and can be defined as characteristic for the observed patterns in the ordination. The most important finding is that nematodes from the LS station differed from all other stations mainly due to increased levels of the FA 16:1ω7c, 14:0, 20:5ω3 and 20:0 (Fig. 4, Table 5). Environmental variables relevant in explaining the observed variation were the volume percentage of silt (19.8%, p = 0.0002), water depth (11.3%, p = 0.05), and volume percentage of fine sand (9.7%, p = 0.02) and coarse sand (9.5%, p = 0.02).

Fig 4. Principal Coordinates (PCO) plot based on the relative fatty acid composition of nematodes from all ANDEEP-SYSTCO stations. The vector overlay indicates FA-specific correlations of more than 60% with one to the first to PCO axes.

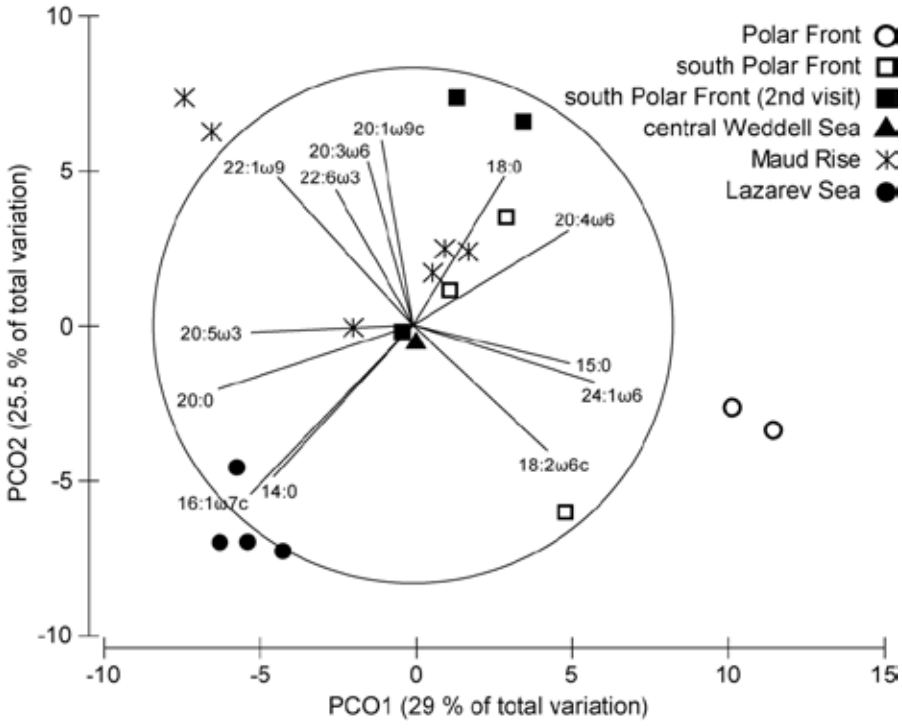


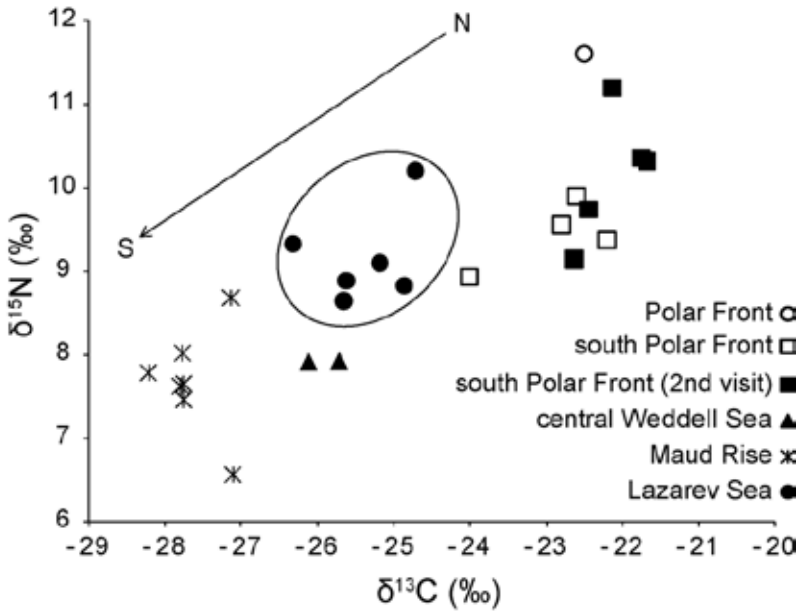
Table 5. Relative concentrations of individual FA, saturated FA (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), FAs of planktonic and bacterial origin, ratios of the FA 16:1ω7/16:0 and 20:5ω6/22:6ω3, the number of nematode individuals analysed per sample, the corresponding dry weight (µg) and lipid weight (µg), and absolute concentrations of total FA of bulk nematodes from all ANDEEP-SYSTCO stations. All concentrations and ratios are expressed as averages and standard deviations of ≥ 2 (pseudo-)replicates.

	PF	sPF	sPF (2nd visit)	cWS	MR	LS
	PS 71 / 90	**PS 71 / 13**	**PS 71 / 85**	**PS 71 / 33**	**PS 71 / 39**	**PS 71 / 17**
12:0	0.7 ± 0.9	0.9 ± 0.2	0.5 ± 0.2	1.7	0.6 ± 0.2	0.4 ± 0.1
14:0	3.3 ± 0.3	5.7 ± 1.7	4.8 ± 0.9	5.1	4.1 ± 0.8	11.0 ± 2.1
14:1ω5c	-	-	1.2 ± 1.1	-	-	-
15:0	1.7 ± 0.9	1.4 ± 0.8	1.0 ± 0.5	0.7	1.0 ± 0.1	0.5 ± 0.2
16:0	7.7 ± 1	11.8 ± 3.9	10.2 ± 3.5	6.3	7.5 ± 3.3	10.6 ± 1.9
16:1ω7c	2.2 ± 0.5	3.5 ± 2.0	4.0 ± 3.4	3.6	3.1 ± 1.3	6.0 ± 0.6
17:0	2.6 ± 2	4.3 ± 0.1	3.0 ± 0.5	2.1	2.5 ± 0.7	1.4 ± 0.3
17:1ω7c	-	-	0.4 ± 0.6	-	-	-
18:0	4.9 ± 0.2	7.4 ± 0.8	5.4 ± 0.6	3.9	5.6 ± 0.9	3.4 ± 0.4
18:1ω9t	13.6 ± 4.4	7.2 ± 2.9	7.0 ± 1.2	8.7	7.7 ± 1.4	10.9 ± 0.8
18:1ω9c	8.6 ± 0.01	8.2 ± 1.4	8.8 ± 1.4	10.1	6.6 ± 0.8	10.1 ± 0.5
18:2ω6t	3.7 ± 5.2	-	-	-	0.9 ± 1.8	-
18:2ω6c	6.0 ± 1.8	1.0 ± 0.6	1.3 ± 0.4	2.9	1.8 ± 0.2	2.1 ± 0.8
20:0	-	0.7 ± 1.1	-	1.6	0.6 ± 1.5	2.5 ± 0.2
20:1ω9t	2.8 ± 1.6	1.9 ± 0.6	1.8 ± 0.5	3.0	2.2 ± 1.0	2.5 ± 0.6
20:1ω9c	3.0 ± 0.1	2.4 ± 1.4	3.3 ± 0.9	3.8	2.8 ± 0.5	2.5 ± 0.8
20:2ω6	3.1 ± 1.6	1.4 ± 0.3	1.2 ± 0.7	3.4	3.7 ± 0.8	1.2 ± 0.2
20:3ω3	-	-	0.1 ± 0.1	0.9	0.2 ± 0.6	0.2 ± 0.3
20:3ω6	-	-	7.6 ± 1.8	-	2.6 ± 3.7	-
20:4ω6	13.7 ± 2.8	8.9 ± 1.3	9.9 ± 4.9	11.2	10.3 ± 3.5	5.8 ± 1.2
20:5ω3	6.9 ± 0.1	9.3 ± 2.2	9.3 ± 3.5	8.6	7.6 ± 0.4	12.3 ± 0.8
22:1ω9	-	1.1 ± 1.0	0.9 ± 0.7	1.5	0.9 ± 0.2	0.8 ± 0.1
22:6ω3	9.7 ± 2.8	19.7 ± 3.8	15.9 ± 2.9	17.5	15.5 ± 5.0	13.8 ± 1.3
24:1ω6	5.9 ± 2.4	3.2 ± 1.9	2.4 ± 0.2	3.4	2.6 ± 0.2	1.8 ± 0.1
∑ SFA	20.8 ± 4.7	32.1 ± 5.8	25.0 ± 4.4	21.3	20.6 ± 3.3	29.9 ± 4.5
∑ MUFA	36.1 ± 3.9	27.6 ± 0.4	29.7 ± 5.2	15.3	25.4 ± 3.6	34.7 ± 2.2
∑ PUFA	43.1 ± 8.5	40.4 ± 6.2	45.3 ± 9.5	34.2	41.2 ± 4.2	35.4 ± 3.9
∑ planktonic	67.6 ± 3.7	59.4 ± 3.0	65.1 ± 3.8	67.0	67.4 ± 3.0	62.5 ± 4.2
∑ bacterial	4.3 ± 2.9	5.7 ± 0.9	4.4 ± 1.4	-	3.5 ± 0.7	2.0 ± 0.4
16:1ω7 / 16:0	0.3 ± 0.03	0.3 ± 0.1	0.4 ± 0.2	0.6	0.5 ± 1.4	0.6 ± 0.1
Nematode (#)	300	300	313 ± 22	543 ± 11	300	300
Dry weight (µg)	53.6 ± 19.0	45.7 ± 12.6	46.5 ± 17.6	141.6 ± 10.8	51.0 ± 14.9	100.6 ± 38.6
Lipid weight (µg)	0.6 ± 0.001	0.7 ± 0.1	1.4 ± 0.8	5.1 ± 3.5	1.4 ± 0.5	2.6 ± 1.3
Lipid (% dwt)	0.01 ± 0.001	1.5 ± 0.1	3.0 ± 1.4	3.6 ± 2.4	2.7 ± 0.9	2.6 ± 1.3

Dual stable isotope signatures

Bulk nematode $\delta^{13}C$ values ranged from -27.7 ± 0.4‰ at the MR station to -22.5 ± 0.03‰ at the PF station (Fig. 5), with significant differences found between all stations except between the stations PF and sPF, PF and sPF (2nd visit), sPF and sPF (2nd visit), and cWS and LS (PERMANOVA, Table 3). $\delta^{15}N$ values ranged from 7.7 ± 0.6‰ at the MR station to 11.6‰ at the PF station (Fig. 5), with significant differences found between all stations expect the stations cWS and MR, sPF and LS, sPF and sPF (2nd visit), and PF and sPF (2nd visit) (Table 3). Both $\delta^{13}C$ and $\delta^{15}N$ values gradually differ along a latitudinal gradient with bulk nematodes being most enriched in ^{13}C and ^{15}N in the northernmost station PF. Only nematodes from the southernmost LS station are an exception on this latitudinal trend and showed intermediate $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 5).

Figure 5. Stable isotope biplot showing $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values of qualitative nematode bulk samples from all ANDEEP-SYSTCO stations, indicating a north-south gradient, with exception of the Lazarev Sea samples (circled).



DISCUSSION

Community structure along the transect

The dominance of nematodes in meiofaunal assemblages is in accordance with other observations in the SO (Herman and Dahms 1992; Vanhove et al. 1995, 2004; Gutzmann et al. 2004), and corresponds to a broad range of deep-sea sediments throughout the world's oceans (Mokievskii et al. 2007). Along the latitudinal ANDEEP-SYSTCO transect, however, nematode relative proportions among the meiofauna as well as meiofaunal densities and nematode standing stock did not consistently decrease with water depth as usually found along continental slopes. We rather suggest that, along the transect, the variable productivity and water depth, that did not increase with distance from land, are interacting driving forces that determine nematode standing stocks. Nevertheless, most of the meiofaunal densities are situated slightly above the World Ocean's regression line provided in Soltwedel (2000) and Wei et al. (2010). Likewise, nematode biomass is relatively high (except at the PF station) compared to areas worldwide at similar depths (Mokievskii et al. 2007).

Despite the broad latitudinal range covered along the ANDEEP-SYSTCO transect (49° S to 70° S, i.e. around 2400 km), relatively little variation is found between the deep-sea nematode communities in terms of generic composition. Although the ordination plot gives an indication of a subdivision into four communities, the low percentage of exclusive genera at each station, together with the dominance of 4 mutual genera between all stations and meiofauna-inherent, high small-scale variability within the stations creates considerable overlap between most of the stations. It is more likely that identification to species level would reveal a clearer separation in subcommunities, since a high degree of species turnover between SO stations was found before (Vermeeren et al. 2004; Fonseca et al. 2006; Ingels et al. 2006; De Mesel et al. 2006). The most remarkable community composition difference among the ANDEEP-SYSTCO stations is however the community at MR which differs from all but the sPF station. Similarly, the composition of several macrofaunal groups at this station showed a peculiar and clearly different composition from other formerly sampled stations in the deep Weddell Sea (Brandt et al. 2011a). Moreover, similar to the nematode assemblages, the composition of polychaetes at MR showed highest affinity to the Polar Front stations (Wilmsen and Schüller 2011). The unique hydrography regime at the seamount MR creates conditions that result in a different frequency, quantity and quality of fresh food compared to other Weddell Sea areas (Abelmann and Gersonde 1991; Wefer and Fischer 1991). However, since MR is under influence of seasonal sea-ice coverage, resulting in a lower organic carbon flux with a different composition compared to the Polar Front region (Wefer and Fischer 1991), the ecological conditions that determine the similarity between the nematode communities of both distant

areas are unknown. The fauna at other seamount habitats is, however, apparently often similar to neighboring areas that fall within organisms' preferred depth ranges (Clark et al. 2010).

The composition of the deep-sea nematode assemblages considered at genus level along the ANDEEP-SYSTCO transect is comparable with deep-sea nematode communities worldwide. Three of the four genera that dominate at all the ANDEEP-SYSTCO stations (*Acantholaimus*, *Halalaimus*, and *Desmoscolex*) are among the dominant genera (> 2% relative abundance) at deep-sea areas worldwide (Vanreusel et al. 2010b). Our results therefore confirm that there is no sign of a distinct Antarctic nematode community at genus level (Vanhove et al. 1999, 2004; Sebastian et al. 2007). In the study of Vanhove et al. (1999) nematode structural and trophic diversity decreased from the shelf break and upper slope (EG(100) = 40, Θ^{-1} = 3.85 to 3.45) towards down slope (EG(100) = 30, Θ^{-1} = 2.94). In comparison, the LS station at a comparable depth as the downslope station in the Eastern Weddell Sea, has a higher structural and trophic diversity (EG(100) = 39, Θ^{-1} = 3.5). The rarefaction index for the LS slope station also agreed well with the index calculated for slopes worldwide (EG(100) = 40), and similarly reduced when the less diverse abyssal communities were taken into account (EG(100) = 34.5 versus 35.5 in Vanreusel et al. 2010b). The Antarctic continental slope (LS) seemed to be the area of highest polychaete diversity too (Wilmsen and Schüller 2011). Two factors that are generally believed to increase diversity at slopes compared to the abyss are higher sedimentation rates and thus enhanced food input, and the reduced stability of the continental slope environment due to the higher eventuality of landslides (Wilmsen and Schüller 2011 and references therein).

Relatively large ranges of feeding types occur among stations along the transect. Epistratum feeders (2A) *sensu* Wieser (1953) dominated at most stations (LS, MR, sPF). Vanhove et al. (1995, 1999, 2004) and Sebastian et al. (2007) observed a dominance of epistratum feeders in Southern Ocean slope and abyssal sediments where the highest input of organic matter was expected based on highest nematode densities. They explained this as a functional adaptation of nematode communities to short-term events of fresh food supply. However, no such link emerges when our feeding type compositions are compared with the labile portion of the carbon flux at each ANDEEP-SYSTCO station, calculated by Sachs et al. (2009).

Temporal variability at the south Polar Front

The deposition of fresh phytodetritus at the sPF station was evidenced by the visual presence of a green fluffy layer, an eight-fold increase in chlorophyll-*a* content in the first half centimeter sediment layer and higher oxygen consumption due to enhanced respiratory activity in the top centimeters, 52 days after the bloom was observed at the sea-surface (Veit-Köhler et al. 2011). In contrast to observations from subtidal North Sea sediments (Vanaverbeke et al.

2004), this event did not induce a rapid increase in total nematode densities and biomass. Our observation is, however, in accordance with previous deep-sea studies (e.g. Gooday et al. 1996; Danovaro et al. 2000) and might support the speculated slower rates of somatic growth and energy requirements in the deep sea (e.g. Giere 2009). Nevertheless, a significantly higher relative abundance of nematodes was found in the top centimeter of the sediment after the bloom settled. It was speculated that this higher relative abundance in the top centimeter indicates migration toward upper sediment layers due to the enhanced availability of organic material (Veit-Köhler et al. 2011). If this is true, then we expect to find evidence for uptake of fresh organic material in, for example, the total fatty acid content of the nematodes, which is addressed later in the discussion.

Nematode feeding ecology

Carbon and nitrogen stable isotope ratios and fatty acid compositions are commonly used to examine the diet of animals and the structure of food webs in marine ecosystems (e.g. Fry 2006 and references therein). Most studies that deal with species level focused on macrofauna organisms that are per definition organisms retained on a 1 mm sieve and therefore with relatively high biomass compared to meiofauna. Since dual stable isotope analysis requires at least 5 µg C or N for reliable detection (Moens et al. 2005a), large numbers of small organisms such as nematodes (0.01 to 0.32 µg C ind.$^{-1}$ in this study) must be grouped to obtain sufficient material for analysis. A lower biomass limit for fatty acid measurements is taxon dependent, temporarily variable, and unknown for deep-sea nematodes. These biomass restrictions impede biochemical analysis at the nematode species or genus level. The fatty acid and dual stable isotope results should, however, be interpreted taking in mind that several studies suggest that nematode communities are not ecologically homogeneous due to taxon-specific trophic requirements (e.g. Wieser 1953; Jensen 1987a; Moens and Vincx 1997).

Fatty acids

Most food web studies in the Antarctic marine ecosystem focus on plankton (Dalsgaard et al. 2003 and references therein). Although benthos is the richest element in the marine food web in terms of numbers of species, their roles and interactions are poorly known (Griffiths 2010). Würzberg et al. (2011a, b) were the first to analyze the FA composition of members of different Antarctic, benthic, polychaete families and orders of peracarid crustaceans, in addition to their potential food sources, including sediment, POM and foraminiferans. This was done on samples collected along the ANDEEP-SYSTCO transect, which allows comparison with the nematode data. Although the bulk analysis on nematodes applied in this study

masks potential genus- or species-specific feeding behavior, it is the first study that obtains information on the spectrum and variety of FA compositions of deep-sea nematodes.

An indication for selective feeding behavior by nematodes is found when comparing nematode FA patterns with FA data obtained for sediment and POM from the stations sPF (2nd visit), cWS, MR and LS by Würzberg et al. (2011b). SFA (16:0, 18:0) and MUFA (16:1ω7, 18:1ω9, 18:1ω7) dominated sediment and POM, while typical indicators of fresh planktonic detritus (PUFA; e.g. 20:5ω3, 22:6ω3) that occur in high proportions in nematodes (34% to 45%), only occur in minor proportions in the sediment and POM (9.4% to 28.7% and 9.1% to 19.2%, respectively; Würzberg et al. 2011b). The high portion of PUFAs in nematodes confirms that marine nematodes can be a high quality food source to predators (Leduc 2009). Furthermore, the generally low lipid content of the nematodes, together with predominance of typical phospholipids of biomembranes (20:5ω3, 22:6ω3 and 16:0; Graeve et al., 1997) indicates that they do not accumulate lipids for energy storage. Additionally, the relatively high percentages of 16:0 and 18:1ω9 in nematodes (6.3% to 22.2%), sediment (9% to 39%) and POM (13% to 40%) points at a potentially unlimited detritivorous feeding behavior, since 16:0 is the main FA in phytodetritus and fecal pellets (Reemtsma et al. 1990; Najdek et al. 1994). High levels of 18:1ω9, up to almost 50% of total fatty acids, in the Antarctic amphipod *Waldeckia obesa* also evidenced feeding on degraded material (Graeve et al. 2001). Moreover, the lack of a significant increase in relative FA content (% of dry weight) in the nematodes after the phytoplankton bloom settled at the seafloor at the sPF station favors the idea of a year-round feeding behavior. The short term response to the settled phytodetritus flux of nematodes inhabiting the sediment surface layer might, however, be underestimated considering a potential dilution effect when pooling together nematodes from five centimeter sediment prior to the analysis. The possibility of feeding throughout the year was also formulated for peracarid crustaceans and polychaetes sampled along the ANDEEP-SYSTCO transect, based on their generally low total FA content (1.0 to 5.1% of individual dry weight; Würzberg et al. 2011a, b). Together with the results of Würzberg et al. (2011a, b), our results therefore support the theory of a permanent food reservoir in the Antarctic deep sea (Mincks et al. 2005; McClintic et al. 2008). Nevertheless, although FA profiles of polar lipids change with dietary composition (Peters et al. 2006), caution has to be taken when interpreting FA patterns of organisms with low lipid reserves. Since selective ingestion of fresh planktonic detritus cannot be distinguished from strong retention of these highly unsaturated FA in metabolic processes (Brett and Müller-Navarra 1997) their proportions could be enhanced (Würzberg et al. 2011b).

Table 6. Relative concentrations of saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated (PUFA) in nematodes, sediment and bottom water POM at the different ANDEEP-SYSTCO stations, based on data obtained in this study and the study of Würzberg et al. (2011b), respectively.

	PF	**sPF**	**sPF (2nd visit)**			**cWS**			**MR**			**LS**		
	Nem	**Nem**	**Nem**	**Sed**	**POM**	**Nem**	**Sed**	**POM**	**Nem**	**Sed**	**POM**	**Nem**	**Sed**	**POM**
∑ **SFA**	20.8	32.1	25.0	35.1	47.9	21.3	25	58.9	20.6	31.2	62	29.9	30.9	45.4
∑ **MUFA**	36.1	27.6	29.7	20.2	19	15.3	50.2	15.4	25.4	40.9	15.3	34.7	24.8	28.2
∑ **PUFA**	43.1	40.4	45.3	11	11.3	34.2	< 6.5	7.9	41.2	< 6	6.7	35.4	10.4	16.3

This study provides a general clue on the importance of planktonic detritus in the diet of deep-sea nematodes in the SO. Plankton derived FA dominate total nematode FA content at all stations with 59% to 68%, while bacterial FA are generally of minor importance (max. 5.7%). This confirms the lack of significant uptake of bacterial carbon in a short-term ^{13}C-enrichment experiment on Arctic deep-sea sediments (Guilini et al. 2010). At the same time it contrasts with studies that suggest that deep-sea nematodes may use bacteria, rather than fresh phytodetritus, based on the natural isotopic shift between sedimentary organic carbon and nematodes, or a minute significant uptake of ^{13}C-labeled diatoms by nematodes under experimental conditions (Goering et al. 1990; Iken et al. 2001; Ingels et al. 2010b).

A significant difference in nematode FA composition between the southernmost station (LS) and all other stations was found. Furthermore, FA proportions of nematodes at MR differed from those at sPF (2nd visit) and PF. Since these differences do not necessarily match differences in nematode genus composition between stations, we expect that the observed variance in FA patterns is rather attributed to variance in the FA composition of available organic carbon sources than to the occurrence of distinct genera or species with different food preferences or metabolic characteristics alone. This is supported by the different grain size variables that explain the compositional variability in nematode community compositions and FA compositions between the stations. The interpretation of FA as quantitative tracers of different organic carbon sources is, however, complicated by many factors. Hydrodynamic processes in the first place, influence temperature, light and nutrient availability, three key factors affecting the FA pattern of the local planktonic primary producers (Dalsgaard et al. 2003). Water depth may also play a role, since lipid compositions of plankton and detritus can be altered considerably during sedimentation and after deposition due to catabolic processes and FA specific diagenetic susceptibility (Graeve et al. 1997 and references therein; Camacho-Ibar et al. 2003 and references therein). In turn, diagenetic alteration depends on environmental factors such as water temperature and dissolved oxygen concentration (Camacho-Ibar et al. 2003 and references therein). The LS station is the shallowest station,

located closest to the shelf, where strong seasonality of ice-coverage and of high carbon fluxes are reported (Bathmann et al. 1991; Fischer et al. 2000; Isla et al. 2009). Both sea-ice and pelagic blooms are largely composed of diatoms superimposed on a background of *Phaeocystis* and dinoflagellates (Dalsgaard et al. 2003 and references therein). *Phaeocystis* spp. in this region are much less rich in ω3-PUFA, which together with a low 16:1ω7/16:0 ratio and an elevated concentration of 18:1ω9 distinguishes them from diatoms (Skerratt et al. 1995). Our results indicate, however, that both 20:5ω3 and 18:1ω9 are high and the ratio of 16:1ω7/16:0 is low, suggesting a mixed diet including both diatoms and *Phaeocystis*, similar to what was found for the planktonic copepod *Calanoides acutus* in the Lazarev Sea (Falk-Petersen et al. 1999). In general, however, it is impossible to say whether nematodes are primary or secondary consumers of phytodetritus since zooplankton may incorporate FA markers from phytoplankton unchanged. The occurrence of 20:1ω9 in nematodes along the ANDEEP-SYSTCO transect might for example point towards the incorporation of remnants of the Antarctic pelagic herbivorous copepods (e.g. *Calanoides acutus* and *Calanus propinquus*) that are known to biosynthesize this MUFA *de novo* as storage product, while it is not a common phytoplankton FA (Hagen et al. 1993). Together with *Metridia gerlachei*, both copepod species accounted for > 50% of the total zooplankton in stations along the Prime Meridian from around 50° southwards (Froneman et al. 2000). Results from several sediment trap studies indicate that within the Marginal Ice Zone, copepods and krill may contribute substantially to total downward particle flux (Pakhomov et al. 2002 and references therein). Nevertheless, it is more generally accepted that outside the periods of sedimentation of ice-algae and phytoplankton blooms, the sinking fecal material of zooplankton, that often contains high concentrations of lipid droplets (Najdek et al. 1994) or undigested phytoplankton cells (Bathmann et al. 1991), largely dominates the downward vertical flux of POC (Pakhomov et al. 2002 and references therein). This may enable nematodes to feed year-round as suggested by their low lipid content.

What complicates the interpretation of FA patterns even more is to what extent typical FA are incorporated unmodified and the species-specific abilities to biosynthesize new FA. Knowledge on such processes in the marine realm is limited to mostly crustaceous zooplankton and fish, and so far unknown for deep-sea benthic invertebrates. Furthermore, the specificity of some FA can be challenged since ambiguity in the origin of FA markers is common. The proportion of 20:4ω6, for example, is high in nematodes at all stations. While it is generally considered to be a phytoplankton derived FA, it also occurs in macroalgae, bacteria, copepods and deep-sea foraminiferans (Nichols et al. 1997; Russell and Nichols 1999; Bühring et al. 2002; Gooday et al. 2002; Suhr et al. 2003; Caramujo et al. 2008). Since it was however detected in only very small proportions in two typical dominant herbivorous copepods in the SO (*Calanoides acutus* and *Metridia gerlachei*, max. 1.3 ± 0.8%; Albers et

al. 1996), and in considerable proportions in foraminiferans at the ANDEEP-SYSTCO stations (max. 20.5 ± 8.4%; Würzberg et al. 2011a), the relatively high proportion found in nematodes (max. 13.7 ± 2.8%) might indicate feeding on foraminiferans, feeding on the food source foraminifers obtain this FA from, or partially by *de novo* biosynthesis. The latter is feasible since the presence of $\Delta 5$ and $\Delta 6$ desaturase and $\omega 6$ PUFA elongase activity in cultured nematodes (*Caenorhabditis elegans* and *Panagrellus redivivus*) has proven to result in *de novo* biosynthesis of 20:4ω6 (Watts and Browse 2002; Schlechtriem et al. 2004). If these speculations are correct and 20:4ω6 in nematodes does not originate from plankton, the proportion of plankton derived FA should be revised. Nevertheless, this fraction would still account for 50.4% to 59.2% of the total FA content of nematodes at all stations.

Dual stable isotope signatures

It is difficult to detect whether nematodes are primary or secondary consumers of plankton based on their fatty acid composition. Complementary trophic analyses by means of dual stable isotope determinations may help to elucidate the food sources. Thereby $\delta^{15}N$ levels of aquatic organisms show an average, stepwise enrichment in ^{15}N of 2.3‰ to 3.4‰ with each step in trophic level, whereas $\delta^{13}C$ levels increase on average by only 0.5‰ at every step and indicate the distance of a consumer from the food base in its trophic system (Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003). However, for this fractionation principle to be useful in identifying food sources and pathways, knowledge on the isotope values of the source materials is essential. Since $\delta^{13}C$ and $\delta^{15}N$ values of surface water phytoplankton were not available from the sampled stations along the ANDEEP-SYSTCO transect, nematode data are interpreted with respect to data for the SO found in literature.

Water depth, and the increase in POM $\delta^{13}C$ and $\delta^{15}N$ occurring with depth due to selective microbial respiration of ^{12}C and ^{14}N (e.g. Saino and Hattori 1980; Biggs et al. 1987; Rau et al. 1991a; Wu et al. 1999), does not explain the observed $\delta^{13}C$ and $\delta^{15}N$ patterns in the deep-sea nematodes. Instead, the signatures of the nematodes are consistent with the reported latitudinal increasing trend of surface ocean $\delta^{13}C_{POC}$ and $\delta^{15}N_{DON/POM}$ from the shelf toward the Subantarctic section of the Atlantic part of the SO (Rau et al. 1991a; Kopczynska et al. 1995; Dehairs et al. 1997; Sigman et al. 1999). One exception to the gradual decline in nematode $\delta^{13}C$ (-21.7‰ to -28.2‰) and $\delta^{15}N$ (11.6‰ to 6.6‰) values was encountered for the community of the southernmost, shallowest station (LS) where $\delta^{13}C$ (-25.4 ± 0.6‰) and $\delta^{15}N$ (9.2 ± 0.6‰) had more enriched values. This likely reflects a dependence on pelagic phytoplankton released from light limitation as the ice edge retreats (typically $\delta^{13}C$ < -30‰; e.g. Wada et al. 1987; Fischer et al. 1991; Rau et al. 1991a; Nyssen et al. 2002) whether or not, and potentially temporarily, including ice algae released from melting sea-ice (typically $\delta^{13}C$ of -20‰ to -25‰; Wada et al. 1987; Bathmann et al. 1991; Fischer et al. 1991; Frazer 1996). Surface $\delta^{15}N$ values

of POM in or closely associated with sea ice in the Weddell Sea ranged from 3‰ to 6‰ (Rau et al. 1991a). Together with the distinct fatty acid patterns, dual stable isotope values of nematodes at the LS station reveal that their diet comprises a distinct component from the other stations along the transect and that this component is highly likely ice algae.

Moreover, based on thermodynamic and biological differences between geographical regions it is clear that the $\delta^{13}C$ and $\delta^{15}N$ values in nematodes reflect surface water variation in POM isotopic signatures rather than differences in trophic levels and food sources of the nematodes. The same pattern was found for benthic copepod communities sampled along the ANDEEP-SYSTCO transect (Veit-Köhler et al. in prep.). Overall, the comparison of $\delta^{13}C$ and $\delta^{15}N$ values of nematodes and surface water POM from literature reveal that at all ANDEEP-SYSTCO stations nematodes are primary, secondary or even tertiary consumers of planktonic detritus (Veit-Köhler et al. in prep.). Future determination of $\delta^{13}C$ and $\delta^{15}N$ values of other benthic organisms and of the increase in POM $\delta^{13}C$ and $\delta^{15}N$ with depth due to selective microbial respiration is the solution to reveal the potential intermediate trophic step between phytodetritus and nematodes.

CONCLUSION

Structural and functional aspects of the deep-sea nematode communities along the ANDEEP-SYSTCO transect differed relatively little. The assemblages are highly comparable to slope and abyssal communities elsewhere in the world in terms of nematode standing stock, diversity and composition on generic level. Changes in nematode relative abundances in sediments of the revisited south Polar Front station, suggested an upward migration towards the settled phytodetritus bloom at the seafloor. Fatty acid compositions in nematodes, evidenced a relatively tight coupling between the planktonic food web and deep-sea nematodes at all stations. They also suggest that nematodes' diets rather reflect the biochemical composition of plankton derived food sources than changes in community compositions and genus- or species specific food preferences or metabolic characteristics alone. Both fatty acid compositions and low fatty acid concentrations additionally reveal that bacteria only contribute to a small extent to the diet of deep-sea nematodes and that nematodes may feed year-round on more degraded or constantly available food sources in the sediment (e.g. fecal pellets, foraminiferans), supplemented by selective feeding on high quality food (phytodetritus) in circumstances of excess fresh labile detritus. A functionally coupled relationship between $\delta^{13}C$ and $\delta^{15}N$ values in nematodes and $\delta^{13}C_{POC}$ and $\delta^{15}N_{PON}$ in the water column was established, reinforcing the bentho-pelagic link that was evidenced by the fatty acids. This study, however, does not exclude the possibility of dissolved organic carbon utilisation, nor feeding on other

organisms like fungi or small protozoans like flagellates and ciliates. Instead, this study should be seen as pioneering work upon which future investigations can build to further elucidate the structure and mechanisms of SO and more general, deep-sea benthic food webs. Future investigations that perform comparative studies on fatty acid compositions and dual stable isotope ratios of both nematodes and their potential food sources might elucidate potential intermediate trophic steps between phytoplankton and nematodes. Biochemical analyses on separate feeding groups *sensu* e.g. Wieser (1953) could help to elucidate whether this classification has practical implications for the role or functionality of deep-sea nematodes. In extreme, the ability of including genus or species discrimination would allow to obtain a higher resolution on the diet compositions and food source partitioning among nematodes.

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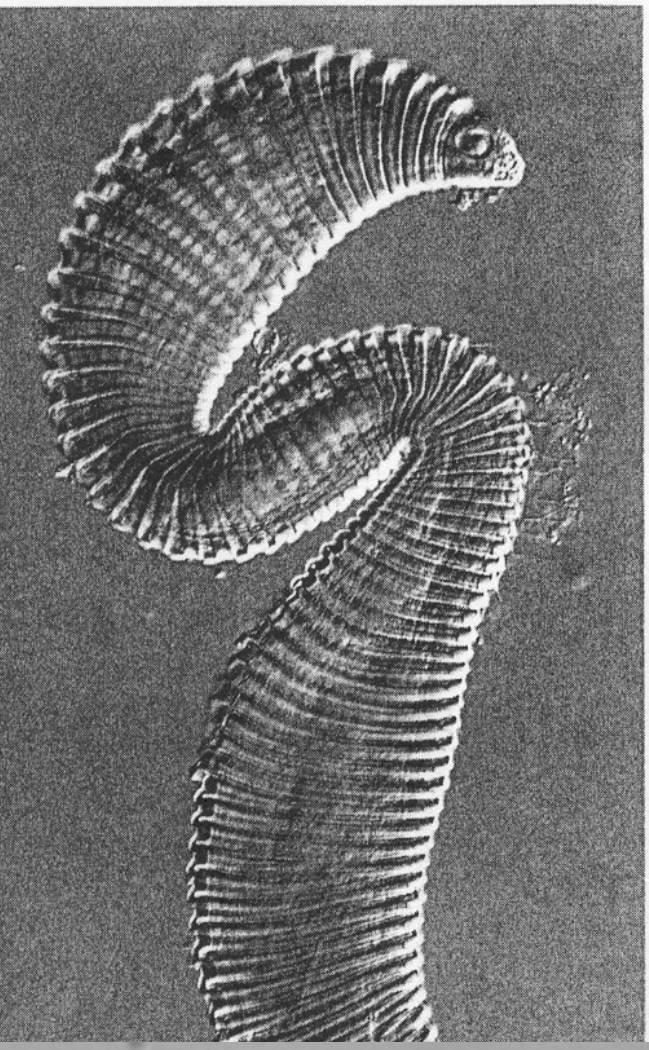
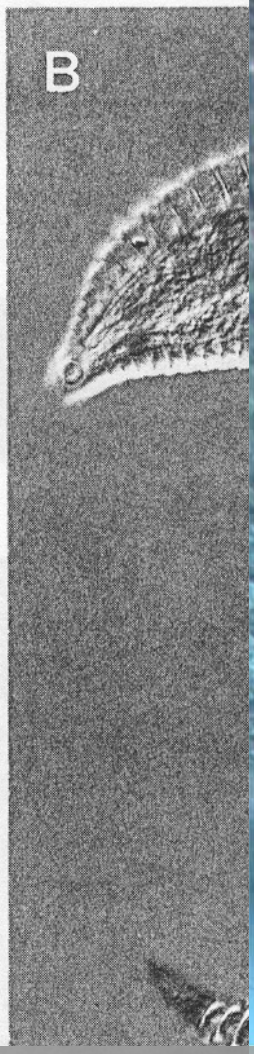
ADDENDUM

Appendix A. Results from main and pairwise one-factor multivariate and univariate PERMANOVA and PERMDISP analyses testing for differences in nematode community composition, and nematode density, relative abundance, biomass, mean individual biomass, and juvenile/adult ratio (0 - 1 cm), respectively, over the top five centimeters of the sediment between the sPF and sPF (2nd visit) station. Significant differences are indicated in bold. ([a]: $0.001 < p \leq 0.05$; [b]: $p \leq 0.001$)

Test	Factor		Nematode community	Nematode density	Nematode relative abundance	Nematode biomass	Nematode mean individual biomass	Nematode juv./ad. ratio in 0 - 1 cm
PERMANOVA	Time	df	1	1	1	1	1	1
		MS	2169.6	**1.3 10^{6} [b]**	$-3.4\ 10^{-12}$	**559.2 [a]**	$7.9\ 10^{-4}$	$3.6\ 10^{-4}$
		Pseudo-F	1.49	4.58	Denominator is 0	9.35	0.10	0.03
	Sediment depth	df	4	4	4	4	4	
		MS	3865.3	3781300	2439.8	1159.5	0.03	
		Pseudo-F	**3.66 [b]**	**33.61 [b]**	**53.43 [b]**	**27.48 [b]**	**3.14 [a]**	
	Re(Time)	df	4	10	10	4	4	
		MS	1456	287860	$-5.3\ 10^{-13}$	59.8	0.008	
		Pseudo-F	**1.38 [a]**	**2.56 [a]**	Negative	1.42	0.91	
	Time x Sediment depth	df	4	4	4	4	4	
		MS	929.8	188480	192.0	202.0	0.002	
		Pseudo-F	0.88	1.68	**4.20 [a]**	**4.79 [a]**	0.26	
	Residuals	df	16	40	40	16	16	4
		MS	1055.1	112510	45.66	42.19	0.009	0.01
	Total	df	29	59	59	29	29	5
PERMDISP	Sediment depth	F	0.49	0.05	0.41	**8.89 [a]**	0.007	
		df 1	1	1	1	1	1	
		df 2	8	8	8	8	8	
	0 - 1 cm: sPF, sPF (2nd visit)	t	1.12	0.20	**2.40 [a]**		1.55	
	1 - 2 cm: sPF, sPF (2nd visit)	t	0.91	2.15	1.05		0.32	
	2 - 3 cm: sPF, sPF (2nd visit)	t	1.26	2.22	2.02		0.05	
	3 - 4 cm: sPF, sPF (2nd visit)	t	0.92	1.28	0.13		0.16	
	4 - 5 cm: sPF, sPF (2nd visit)	t	0.90	2.10	1.37		1.37	

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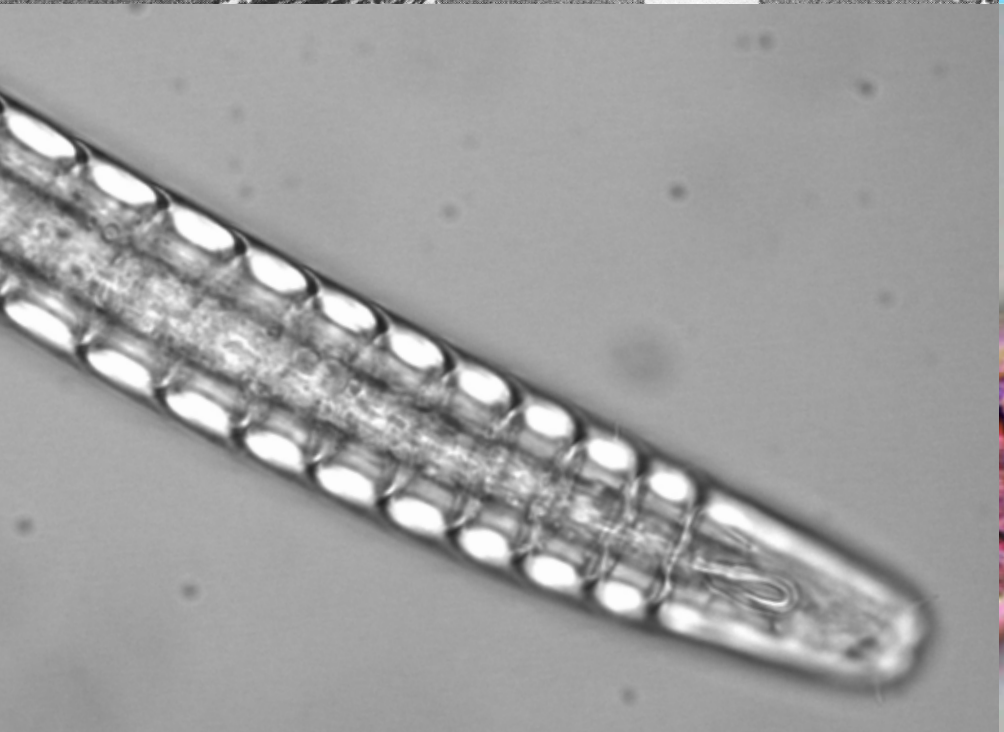

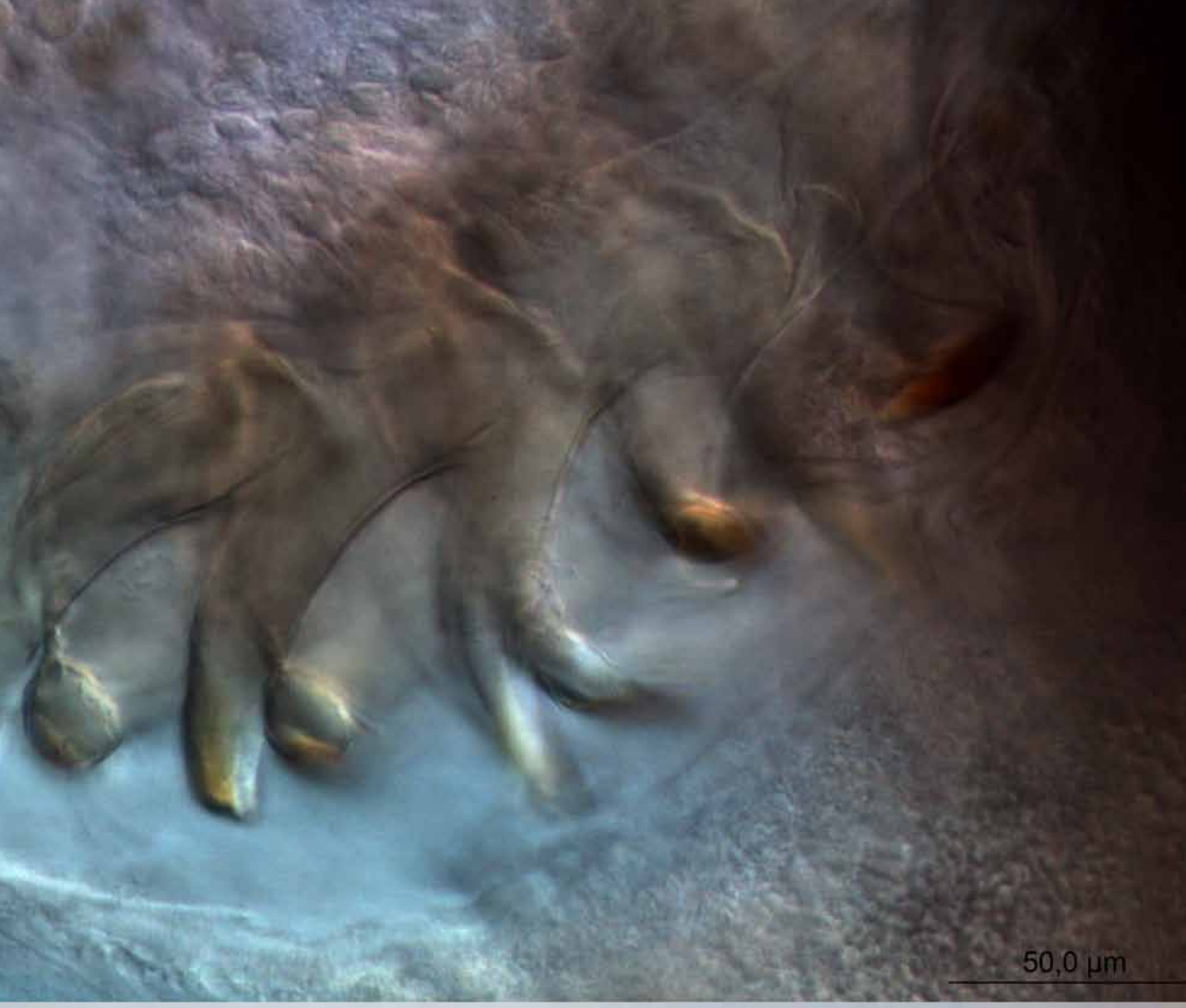
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Cold seep and oxygen minimum zone associated sources of margin heterogeneity affect benthic assemblages, diversity and nutrition at the Cascadian margin (NE Pacific Ocean)

Slightly modified from the publication in press:

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ABSTRACT

Hydrate Ridge (HR), located on the northeastern Pacific margin off Oregon, is characterized by the presence of outcropping hydrates and active methane seepage. Additionally, permanent low oxygen conditions overlie the benthic realm. This study evaluated the relative influence of both seepage and oxygen minima as sources of habitat heterogeneity and potential stress-inducing features on the bathyal metazoan benthos (primarily nematodes) at three different seep and non-seep HR locations, exposed to decreasing bottom-water oxygen concentrations with increasing water depth. The nematode seep communities at HR exhibited low diversity with dominance of only one or two genera (*Daptonema* and *Metadesmolaimus*), elevated average individual biomass and $\delta^{13}C$ evidence for strong dependance on chemosynthesis-derived carbon, resembling deep-sea seeps worldwide. Although the HR seep habitats harbored a distinct nematode community like in other known seep communities, they differed from deep-sea seeps in well-oxygenated waters by sharing the dominant genera with the surrounding non-seep sediments overlain by oxygen-deficient bottom water. The homogenizing effect of the oxygen minimum zone on the seep nematode assemblages and surrounding sediments was constant with increasing water depth and concomitant greater oxygen-deficiency, resulting in a loss of habitat heterogeneity.

Keywords: Cascadian subduction zone · Hydrate Ridge · Cold seep · Oxygen minimum layer · Meiobenthos · Nematoda · Macrobenthos · Habitat heterogeneity

INTRODUCTION

Cold-seeps are geochemical features, found worldwide on tectonically active and passive continental margins (Sibuet and Olu 1998). They are formed where subsurface interstitial fluids, rich in reduced chemicals from biogenic or thermogenic origin (mostly methane and sulfide), are released at the sediment surface as a result of a variety of processes such as tectonic activity, differential compaction of organic-rich sediments, gas hydrate dissociation and subsurface salt migration (Cordes et al. 2010). Therefore cold-seep ecosystems appear in a variety of geomorphic and biological forms on the seafloor and create a diverse suite of habitats for both endemic seep organisms and more opportunistic colonists (Levin et al. 2003; Cordes et al. 2010). Together with water depth and age of the geologic features, habitat heterogeneity created by the intensity and volume of fluid flow, and methane and sulfide concentrations and fluxes, determines species richness and density of the inhabiting fauna (Cordes et al. 2010). While chemo-autotrophic bacteria that thrive on hydrogen sulfide form mats at sites of active seepage, relatively few taxa have the ability to use or

to tolerate these physico-chemical conditions (Levin et al. 2003). Several megafaunal seep-specialists are adapted to survive in these reduced habitats by carrying endosymbionts and/or by having sulfide-binding or -oxidizing capabilities (e.g. siboglinid polychaetes, cladorhizid sponges, bathymodiolin mussels, and vesicomyid, lucinid, solemyid, and thyasirid clams). This successful exploitation of chemosynthetic derived energy is often evidenced by highly increased levels of density and biomass of these few sessile or sedentary taxa. Their presence also adds structural complexity to the seep sediments which increases habitat heterogeneity and supports many smaller fauna living associated with them (Levin 2005). Overall, the aggregations of symbiotic foundation species and associated taxa at cold seeps are considered as exceptions on the general trends of high local metazoan diversity and evenness, and low metazoan density and biomass that are found in most of the deep sea (Levin 2005).

In addition to cold seeps, oxygen minimum layers present at intermediate water depths (ca. 100 - 1000 m), contribute to habitat heterogeneity along continental margins worldwide. This hydrographic feature persists over geological time scales and occurs where upwelling leads to high surface productivity, oxygen-depleted source waters are present, and water stability is imposed by a strong pycnocline that induces accumulation of settling organic detritus (Levin 2003; Gooday et al. 2010). Typically, O_2 concentrations drop below 0.5 ml l^{-1}. As reduced bottom-water oxygen concentrations enhance preservation of organic matter from the surface (Cowie and Hedges 1992), increased concentrations of labile organic matter and phytodetrital food are found in sediments where the OMZ intersects with the seafloor (often > 3%, up to 20.5% organic carbon; Levin 2003; Gooday et al. 2010). Strong zonation of benthic communities occurs in conjunction with varying bottom- and pore-water oxygen concentrations and associated gradient in organic-matter input that change with water depth (e.g. Levin et al. 1991, 2000, 2003, 2009; Wishner et al. 1990, 1995; Levin and Gage 1998; Neira et al. 2001). Macro- and megafauna communities inhabiting OMZ sediments are generally distinct from the well-oxygenated surrounding margins in having severely reduced densities, biomass and diversity (Wishner et al. 1990; Levin and Gage 1998; Smith et al. 2000; Levin et al. 2001; reviewed in Levin 2003; Sellanes et al. 2010). Although total meiobenthic biomass and abundance often follow the same pattern as mega- and macrofauna, both protozoan and metazoan meiofauna are generally less affected by low oxygen conditions (Levin et al. 1991; Bernhard et al. 2000; Cook et al. 2000; Gooday et al. 2000; Neira et al. 2001; Gooday et al. 2009; Veit-Köhler et al. 2009). Among the metazoan meiofauna, deep-sea nematodes seem very tolerant to low oxygen concentrations (Levin et al. 1991; Moodley et al. 1997; Cook et al. 2000) and typically occur at higher densities where O_2 concentrations are lowest in OMZs compared to other taxa that are unable to tolerate low oxygen concentrations such as harpacticoid copepods and nauplii (Hicks and Coull 1983; Murrell and Fleeger 1989;

Levin et al. 1991; Giere 2009; Neira et al. 2001; Levin et al. 2009b). If their elongated body-length and therefore higher surface area-to-volume ratio could be an adaptation to low oxygen partial pressure as suggested before (Jensen 1986, 1987b; Schiemer et al. 1990; Soetaert et al. 2002), morphometric changes might occur along an oxygen-deficiency gradient.

Given the widespread occurrence of both seeps and OMZs on East Pacific margins it is not surprising to find a considerable number of seeps that coincide with OMZs (Levin et al. 2010a; Sellanes et al. 2010). Hydrate Ridge (HR) is such a cold seep ecosystem on the Northeastern Pacific margin off Oregon where permanent low oxygen conditions overlay the benthic realm. The OMZ extends from about 650 to 1100 m water depth, with a minimum around 800 m (< 0.2 ml l^{-1}) (Helly and Levin 2004) whereas high seepage occurs between 600 and 900 m water depth. Key seep habitats at HR include mats of the filamentous sulfide oxidizing *Beggiatoa* bacteria, *Calyptogena* vesicomyid clam beds and surrounding *Acharax* solemyid bivalve beds (Sahling et al. 2002). Levin et al. (2010a) hypothesized that the effect of oxygen minimum stress would act to minimize seep-induced heterogeneity in macrofauna assemblages at a southern HR location (770 m water depth). In contrast to what was hypothesized, the species overlap between the HR seep habitats and the non-seep sediments was less than in more oxygenated realms overlaying the 500-m Eel River (ER) seeps off California (Levin et al. 2010a). However, stronger fluid fluxes and higher sulfide levels at HR seeps compared to the ER seeps may have induced these results (Levin et al. 2010a). Here we investigate the structuring effect of the OMZ interacting with seep effects on benthic assemblages along a bottom-water oxygen gradient at HR only.

On the margin along HR, increasing sediment carbon concentrations were found with increasing depth within the OMZ (500m: 0.9% C, 770m: 1.99% C, 800m: 2.14% C; Levin et al. 2010a; Valentine et al. 2005). This is likely the result of reduced detrital remineralization due to lower oxygen concentrations with increasing depth. The OMZ might therefore also affect the functional properties of the seep-associated fauna in the sense that with depth the contribution of allochtonous organic carbon might increase in proportion to the autochthonous chemosynthetic-derived carbon input. When seep-associated fauna depend largely on methane-derived carbon, either by carrying methane- or sulfide-oxidizing endosymbionts or by feeding directly or indirectly on chemoautotrophic methane oxidizers (aerobic bacteria and anaerobic Achaea with associated sulfate-reducing bacteria), their tissues will reflect the thermo- or biogenic $\delta^{13}C$ methane signal ($\delta^{13}C$ of -50 to -110‰, e.g. Whiticar et al. 1986). However, as most seep-associated fauna probably use multiple food sources, the interpretation of isotopic signatures is complicated. To yield at least upper and lower estimates of methane-derived carbon in organisms, two-end member linear mixing models with single isotope measures can be used (e.g. Levin and Michener 2002; MacAvoy et al. 2002; Carlier et al. 2010; Demopoulos et al. 2010; Thurber et al. 2010).

Our study evaluates the relative influence of both seepage and oxygen minima as sources of habitat heterogeneity and potential stress-inducing features on the bathyal benthic community. We focus on the nematode community composition, diversity, morphometry, biomass, density and nutrition and compare the latter with macrofauna data. Therefore three habitat types (clam beds, microbial mats and nearby non-seep margin sediments) were studied at three HR locations categorized according to increasing depth and decreasing bottom-water oxygen concentrations (500 - 600 m, 0.7 O_2 ml l^{-1}; 700 - 800 m, 0.2 to 0.3 O_2 ml l^{-1}; 800 - 900 m, $\leq$ 0.2 O_2 ml l^{-1}; Levin et al. 2010a). This study sought to answer the following questions: (1) do the seepage habitats at HR harbor a distinct nematode community compared to the non-seep margin sites and other known seepage sites worldwide? (2) Does the increased habitat complexity created by clam beds affect the nematode community distribution in the sediment, resulting in an increase in biodiversity compared to the microbial mats? (3) Does the OMZ homogenize the nematode composition in such a way that seep-related heterogeneity is more pronounced at more oxygenated water depths? (4) Do water depth and co-varying O_2 bottom water concentrations create a bathymetric faunal zonation at the non-seep sites? (5) What is the importance of methane-derived carbon for nematodes and how variable is trophic specialization among the studied locations and habitats? (6) How do the nematode nutritional trends compare to those observed for macrofauna at these sites?

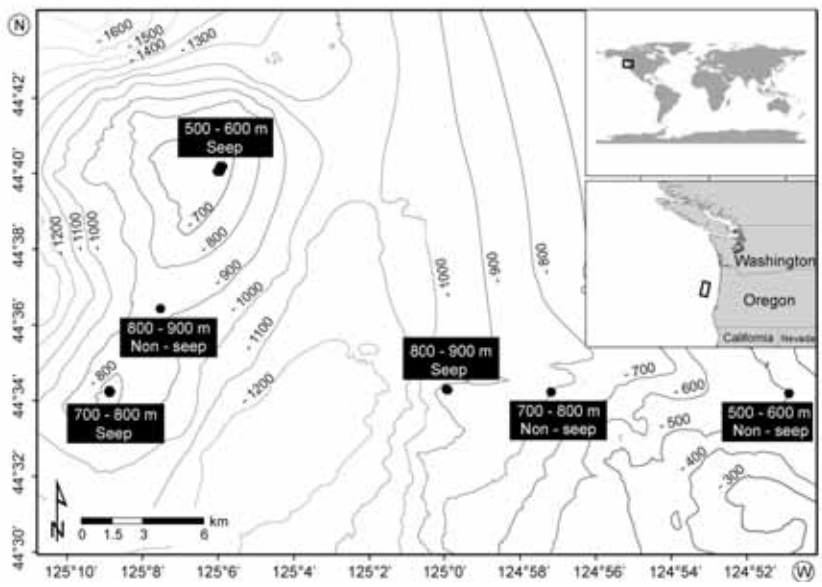
MATERIAL AND METHODS

Site characteristics

Samples for this study were collected at a seepage location in the NE Pacific Ocean, 90 km offshore Oregon (referred to as Hydrate Ridge) and on the continental margin within 30 km of the seepage location, at comparable depths (Fig. 1). Hydrate Ridge (HR) is one of several ridges along an accretionary prism of the Cascadian subduction zone, where the Juan de Fuca plate is subducting under the North American Plate. The ridge, characterized by the presence of outcropping gas hydrates and methane venting cold seeps, is about 25 km long and 15 km wide, and has a northern and southern peak. Three regions are distinguished due to their extensive methane seepage: the southern summit around 770 m, the northern summit at around 600 m and the eastern deepest site at around 880 m water depth. Gas hydrate dissociation is the main source of the methane enrichment in the seeping fluids. The high sulfide fluxes produced by the anaerobic oxidation of seeping methane (e.g. Boetius et al. 2000; Knittel et al. 2003; Treude et al. 2003), and the presence of bacterial and macrofaunal chemosynthetic communities that form key habitat patches at HR have been well documented (e.g. Kulm et al. 1986; Juhl and Taghon 1993; Sahling et al. 2002; Sommer

et al. 2002; Levin et al. 2010a). Studies on the associated meiofaunal communities were restricted to the level of phyla (Sommer et al. 2002, 2003, 2007). The habitat types of interest for this study are the microbial mats that are primarily composed of white, filamentous sulfide-oxidizing bacteria such as *Beggiatoa* sp. and the clam beds formed by the vesicomyids *Calyptogena pacifica* and C. *kilmeri* that harbor sulfide-oxidizing symbionts. Both habitat types occur in patches at scales of meters to tens of meters covering the sea floor (Sahling et al. 2002; Levin et al. 2003). In the HR microbial mats, H_2S concentrations reach over 28 mM within the top 5 cm of the sediment, while in clam beds, net fluid flux rates are lower and H_2S concentrations often only reach the same (or lower) levels below 5 - 10 cm down the sediment (Sahling et al. 2002; Valentine et al. 2005; Sommer et al. 2007).

Figure 1. Bathymetric map of the Hydrate Ridge study location with indication of the three sampled seep locations, each including clam beds and microbial mats, and the three sampled non-seep reference locations; with indication of water depth.



Sampling

Samples to study the meiobenthos were collected in July - October 2006 and August 2010 with the submersible *ALVIN* aboard the RV *Atlantis*. Seep samples were taken with push cores (6.4 and 8.3 cm internal diameter), while sampling of margin sediments, ca. 14 - 30 km further off-seep site, was conducted by multicorer (9.6 cm internal tube diameter). We will further refer to the different types of sampled habitats as 'clam bed', 'microbial mat' and 'non-seep', and classify them according to depth zones to facilitate comparison along an increasing stress gradient of oxygen depletion in the water column (500 - 600 m, 0.7 O_2 ml l^{-1}; 700 - 800 m, 0.2 to 0.3 O_2 ml l^{-1}; 800 - 900 m, $\leq$ 0.2 O_2 ml l^{-1}; Levin et al. 2010a) (Table 1). To obtain a more complete view on feeding habits of the benthic community, we included $\delta^{13}C$ isotope data of macrofauna obtained from samples collected with push cores (6.4 and 8.3 cm internal diameter) and occasionally a slurp, suction or scoop device in clam beds and microbial mats and by multicorer (9.6 cm internal diameter) for margin sediments off seeps (Table 2). Macrofauna samples were collected from the same areas as the meiofauna in August 2005 aboard RV *Western Flyer* (with ROV *Tiburon*) and in July and October 2006 aboard the RV *Atlantis* (with submersible *ALVIN*).

Table 1. Study site location, habitat type, core code, date and depth of sampling, coordinates of exact sampling location, sampling device, surface area of meiofaunal samples and the lower mesh used for sieving (the star marks the samples where the 5 - 10 cm sediment layer was sieved on a 300 µm mesh).

Location		Habitat	Core code	Date	Depth (m)	Latitude N	Longitude W	Sampling device (surface area)	Lower mesh
500 - 600 m	HR North	Microbial mat	TC 16	04/08/2010	600	44° 40.026′	125° 5.920′	Push core (32.2 cm²)	32 µm
	HR North	Microbial mat	TC 23	04/08/2010	600	44° 40.026′	125° 5.995′	Push core (32.2 cm²)	32 µm
	HR North	Clam bed	TC 6	21/07/2006	602	44° 40.169′	125° 5.919′	Push core (32.2 cm²)	32 µm
	HR North	Clam bed	TC 53	21/07/2006	602	44° 40.158′	125° 5.855′	Push core (54.1 cm²)	42 µm
	Margin	Non-Seep	MC 3C	07/2006	491	44° 34.163′	124° 50.864′	Multicorer (72.4 cm²)	42 µm*
	Margin	Non-Seep	MC 2C	07/2006	494	44° 34.163′	124° 50.865′	Multicorer (72.4 cm²)	42 µm*
700 - 800 m	HR South	Microbial mat	TC 23	17/07/2006	772	44° 34.204′	125° 8.841′	Push core (32.2 cm²)	32 µm
	HR South	Microbial mat	TC 51	18/07/2006	774	44° 34.225	125° 8.842′	Push core (54.1 cm²)	42 µm
	HR South	Clam bed	TC 4	20/07/2006	772	44° 34.234′	125° 8.853′	Push core (32.2 cm²)	32 µm
	HR South	Clam bed	TC 20	27/09/2006	774	44° 34.188′	125° 8.849′	Push core (32.2 cm²)	32 µm
	Margin	Non-Seep	MC 2A	19/07/2006	797	44° 34.201′	124° 57.150′	Multicorer (72.4 cm²)	42 µm*
	Margin	Non-Seep	MC 2C	19/07/2006	797	44° 34.201′	124° 57.150′	Multicorer (72.4 cm²)	42 µm
800 - 900 m	HR East	Microbial mat	TC 53	23/07/2006	880	44° 34.257′	124° 59.883′	Push core (54.1 cm²)	32 µm
	HR East	Clam bed	TC 9	23/07/2006	880	44° 34.279′	124° 59.921′	Push core (32.2 cm²)	32 µm
	Margin	Non-Seep	MC 2A	28/09/2006	887	44° 36.402′	125° 07.501′	Multicorer (72.4 cm²)	42 µm
	Margin	Non-Seep	MC 4A	30/09/2006	886	44° 36.402′	125° 07.501′	Multicorer (72.4 cm²)	42 µm

Table 2. Study site location, habitat type, depth of sampling, number of samples for associated macrofaunal $\delta^{13}C$ measurements, and number of $\delta^{13}C$ measurements per taxon. The number of samples are given per sampling campaign (A: August 2005, B: July 2006, C: October 2006).

Location		Habitat	Depth (m)	Samples for $\delta^{13}C$ (n)			$\delta^{13}C$ measurements per taxon (n)			
				A	B	C		A	B	C
500 - 600 m	HR North	Microbial mat	588 - 602	3	3	0	Gastropoda	2	3	0
							Polychaeta	3	9	0
	HR North	Clam bed	590 - 602	6	7	0	Bivalvia	0	2	0
							Crustacea	1	13	0
							Gastropoda	0	3	0
							Nemertea	0	1	0
							Olygochaeta	5	0	0
							Ophiuroidea	1	0	0
							Polychaeta	15	21	0
	Margin	Non-seep	500 - 524	0	1	3	Aplacophora	0	1	0
							Bivalvia	0	2	0
							Crustacea	0	0	1
							Ophiuroidea	0	1	0
							Polychaeta	0	2	12
700 - 800 m	HR South	Microbial mat	770 - 800	4	3	6	Bivalvia	0	0	3
							Gastropoda	1	3	8
							Polychaeta	6	7	17
	HR South	Clam bed	770 - 800	3	9	9	Bivalvia	0	3	4
							Crustacea	0	2	1
							Gastropoda	0	3	2
							Oligochaeta	0	1	8
							Polychaeta	9	33	38
	Margin	Non-seep	800	0	0	4	Aplacophora	0	0	2
							Cnidaria	0	0	1
							Crustacea	0	0	2
							Nemertea	0	0	1
							0phiuroidea	0	0	1
							Polychaeta	0	0	7
							Sipuncula	0	0	1
800 - 900 m	HR East	Microbial mat	872 - 890	4	2	0	Gastropoda	2	0	0
							Polychaeta	3	2	0
	HR East	Clam bed	872 - 880	2	1	0	Gastropoda	0	1	0
							Polychaeta	6	12	0
	Margin	Non-seep	800	0	2	0	Bivalvia	0	2	0
							Cumacea	0	2	0
							Ophiuroidea	0	1	0
							Polychaeta	0	4	0

Sample processing and analytical procedures

The samples destined for meiofaunal identification were vertically sectioned at 1 or 2 cm intervals to 5 cm, and from 5 to 7 and 7 to 10 cm depth on board. All sample fractions were unsieved prior to preservation except for the multicorer samples where in some cases the 5 - 10 cm fraction was sieved on a 0.3 mm mesh. Samples were preserved in 8% buffered formalin. In the laboratory of the SCRIPPS Institute of Oceanography part of the samples were sieved on nested 0.3 mm and 42-µm mesh in order to extract the macrofauna from the 0.3 mm fraction. The residue (including meiofauna) was recombined with the finer fraction and stored in 8% buffered formalin again. In the laboratory of the Marine Biology research group at the Ghent University all meiofaunal samples were sieved on a 1-mm and 32-µm mesh. The fractions retained on the 32-µm mesh sieve were centrifuged three times with the colloidal silica polymer LUDOX HS 40 (Heip et al. 1985) and rinsed with tap water. The extracted fraction was preserved in 4% buffered formalin and stained with Rose Bengal. All metazoan meiobenthic organisms were classified at higher taxon level and counted under a stereoscopic microscope. When possible a minimum of 100 nematodes per sediment layer were randomly handpicked with a fine needle. These nematodes were transferred to glycerine (De Grisse I, II and III; Seinhorst 1959), mounted on glass slides and identified to genus level. Based on mouth morphology, all identified individuals were grouped into four groups of feeding types according to Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A) and predators/scavengers (2B). By using a Leica DMR compound microscope and Leica LAS 3.3 imaging software nematode length (L, filiform tail excluded) and maximal width (W) were measured for the first cm fraction in one replicate of each habitat at every location. Nematode biomass was then calculated with Andrassy's formula (Andrassy 1956):

$$\text{wet weight } (\mu g) = L\ (\mu m) \times W^2\ (\mu m) / 1.6\ 10^6$$

and a dry-to-wet-weight ratio of 0.25 was assumed (Heip et al. 1985). Nematodes were pooled into biomass and morphometric classes (length and width) on untransformed geometric scales. Morphometric size spectra were created by plotting nematode relative abundances against geometric classes of length (µm) and width (µm). Additionally body length, body width, oesophagus length and tail length were measured of two specific dominant genera (five male individuals each) to determine the De Man ratios (a = body length / body width, b = body length / oesophagus length, c = body length / tail length; De Man 1880).

After extracting nematodes for identification, a minimum of 75 nematodes were handpicked for stable isotope analyses with a fine needle from the top two centimeter fraction and rinsed

in MilliQ water to remove adhering particles, before being transferred to a drop of MilliQ water in 2.5 × 6 mm aluminium (Al) cups. Nematodes specimens were picked in bulk or when present in sufficient numbers, genus specific, i.e. *Daptonema*, and *Metalinhomoeus* together with *Linhomoeus* since they could not be distinguished under the stereoscopic microscope. The Al cups were first preheated at 550°C in order to remove all exogenous organic carbon. When present in the samples, large filamentous bacteria were also isolated in Al cups. The cups with nematodes and bacterial filaments were then oven-dried at 60°C, pinched closed and stored in Multi-well Microtitre plates under dry atmospheric conditions until analysis. A PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20 - 20 isotope ratio mass spectrometer (UC Davis Stable Isotope Facility, California) was used to measure the carbon stable isotope ratios and carbon content. During this procedure a minimal He dilution was applied for the low biomass nematode samples. Immediate separation of bulk macrofaunal samples and further preparation for carbon stable isotope analysis was feasible on board and performed as described in detail by Levin and Michener (2002). Analyses were conducted on a Finnigan Conflow 2 continuous flow system and a Fisons NA 1500 elemental analyzer coupled to a Finnegan Delta S isotope ratio mass spectrometer at Boston University or on a continuous flow PDZ Europa 20/20 isotope ratio mass spectrometer at UC Davis. While colloidal silica gel Ludox did not affect the $\delta^{13}C$ signal of shallow water nematodes (Moens, unpubl. data) and Bengal Rose had no significant effect on $\delta^{13}C$ values of shallow water foraminifera (Serrano et al. 2008), buffered 10% formalin preservation resulted in a mean shift in carbon isotopes of $\leq$ -2.0‰ relative to freezing as a control for several macrofaunal invertebrate taxa (Fanelli et al. 2010).

Isotope ratios are expressed as $\delta^{13}C$ in units per mil (‰), according to the standard formula $\delta^{13}C = [R_{sample} / R_{VPDB} - 1] \times 10^3$, where R is the ratio of $^{13}C/^{12}C$, R_{VPDB} is Vienna Pee Dee Belemnite as reference standard, and $R_{sample} = [(\delta^{13}C_{sample} / 1000) + 1] \times R_{VPDB}$. A more positive δ value is isotopically enriched, meaning that proportionally more of the heavy stable isotope is present. The contribution of methane-derived carbon (MDC) in the macrofaunal and nematode carbon pool of each location and habitat was estimated with a single isotope, linear mixing model using two sources (Fry and Sherr 1984). The formula used was:

$$F_m = (\delta_f - \delta_{POC \text{ or } SOB}) / (\delta_m - \delta_{POC})$$

where F_m is the fraction of MDC, and δ_f, δ_m, δ_{POC} and δ_{SOB} are the carbon isotope signatures of fauna, methane, particulate organic carbon (POC) and sulfide-oxidizing bacteria (SOB), respectively. For each analyzed sample a maximum and minimum estimate of MDC was calculated based on the use of respectively the δ_{POC} or δ_{SOB} term in the model. F_m was calculated for each macrofaunal individual and bulk nematodes sample, and then averaged within a higher taxon group to generate values for each habitat and location (± standard

deviation). Similar to Levin and Michener (2002) we used methane $\delta^{13}C$ values of -65‰, measured by Suess and Whiticar (1989). Knowledge on potential spatial and temporal variability of methane $\delta^{13}C$ values is lacking and therefore impedes the use of a range of values. The $\delta^{13}C$ of SOB was based on the average value of microbial mat bacteria measured in this study and the study of Levin and Michener (2002) (i.e. -33.2 ± 5.6‰). The $\delta^{13}C$ of POC was based on the average value for non-seep animals in each location (500 - 600 m: -19.1‰, 700 - 800 m: -20.2‰, 800 - 900 m: -18.7‰).

Data analysis

Statistical tests on nematode data were performed on a maximum of two replicate samples per habitat type per location. Prior to the analyses nematode data were grouped into three sediment depth layers (0 - 1, 1 - 2 and 2 - 5 cm), taking into account the individual counts per depth layer. To test if the nematode community composition was significantly different between samples with different attributes, a non-parametric permutational ANOVA (PERMANOVA) was performed. Bray-Curtis similarity was used as resemblance measure on standardized and square root transformed genus abundance data. The model had an unbalanced fully crossed four factor design and included the fixed categorical factors 'location' (Lo), 'habitat' (Ha) and 'sediment depth' (De), with the random 'replicate' (Re) factor nested in 'Ha', since data from different depth layers from a single replicate core are not fully independent, and all interaction terms. Calculation of the Pseudo-F ratio and P value ($\alpha = 0.05$) was based on 9999 permutations of the residuals under a reduced model. A restricted set of appropriate a posteriori pair-wise tests was conducted where the interaction terms were found to be significant and where a PERMDISP test confirmed the homogeneity of multivariate sample dispersions. Where only a restricted number of unique permutations was possible (mainly in pairwise tests), p-values were obtained from Monte Carlo samplings (Anderson and Robinson 2003). To visualize the PERMANOVA results a non-metric multidimensional scaling (MDS) plot was constructed based on the distance between centroids measure of nematode abundance data per cm sediment depth for the three different habitats at each location. To reveal the variability among and between sample groups a SIMPER analysis was performed on standardized and square root transformed abundance data. Additional multivariate and univariate PERMANOVA analyses (based on Bray-Curtis and Euclidean distance resemblance measure, respectively) with variations on the number of factors in the design as described above were used to test for differences in nematode trophic composition and nematode densities, meiofaunal densities, meiofaunal taxon richness and nematode dominance, respectively. A univariate, two fixed factor design for each location was used to test for difference in $\delta^{13}C$ values between higher taxa at the different habitats (factors 'Ha' and 'Ta' for Taxon; i.e. bacteria, macrofauna and nematodes). A combined analysis

with as extra factor 'Lo' would have been biased by the strongly unbalanced design provoked by the low number of taxa sampled at the deepest site. Only for nematode, polychaete and gastropod data was replication sufficient at each location to test for differences in $\delta^{13}C$ signatures and MDC with water depth (factor 'Lo').

To asses nematode structural and functional diversity, several indices that are variably dependent on relative genera abundances, were generated. The Hill's indices (H_0 = number of genera, H_1, H_2, H_{inf}; Hill 1973), Expected number of Genera for 51 individuals (EG(51); Sanders 1968) and trophic diversity (Θ^{-1}; Heip et al. 1988) were calculated on summed (0 - 5 cm) and standardized abundance data, taking into account the densities per sediment layer. To detect differences in nematode diversity and biomass univariate PERMANOVA analyses were applied with pairwise tests a posteriori. These data required a crossed, two factor design with fixed factors 'Ha' and 'Lo'. Euclidean distance based resemblance matrices were used. Additional rarefaction curves of the Expected number of Genera (EG(n)) were generated from untransformed data and cut off where the maximum estimated number of genera was reached for one of the replicates. Additionally an estimate was made for the number of nematode families. All analyses were performed within PRIMER v6 with PERMANOVA+ add-on software (Clarke and Gorley 2006; Anderson et al. 2008). The STATISTICA 7 software was used to test whether the frequency distributions of nematodes in morphometric classes in clam beds differed significantly from the non-seep sites with a chi-square (χ^2) goodness-of-fit test. Results expressed as means are accompanied by standard deviations.

RESULTS

Meiofaunal densities

A total of 19 higher meiofauna taxa were observed, while taxon richness varied from 3 to 11 taxa per sample (Appendix A). Meiofaunal densities in the sediment layer from 0 - 10 cm ranged from 44 ind. 10 cm^{-2} in a microbial mat sample at 800 - 900 m to 1237 ± 568 ind. 10 cm^{-2} in non-seep margin samples at 700 - 800 m depth (Table 3). Nematodes comprised the dominant taxon in all habitats, ranging from 80 ± 17% in clam beds at 500 - 600 m to 98 ± 0.2% in non-seep sites at 700 - 800 m (Table 3, Appendix A). They were followed in decreasing order of appearance by adult Copepoda (max. 11.2%), Nauplii (max. 6%), meiofaunal sized Polychaeta (max. 4.8%) and Oligochaeta (max. 2.3%) and other taxa with relative abundances less than 1% (Appendix A). Meiofaunal taxon richness and nematode dominance varied strongly between replicates and did therefore not differ significantly between habitats or locations (Main tests, P(perm) ≥ 0.29; Table 4). A habitat effect was

detected for meiofauna densities (Main test, P(perm): 0.009; Table 4), with significant differences found between microbial mats and clam beds (Pairwise test, P(perm): 0.003) and microbial mats and non-seep samples (Pairwise test, P(perm): 0.015); microbial mats harbored the lowest densities.

Nematode densities were highest in the top two cm of the sediment and gradually decreased with depth (Fig. 2). Relative abundance in the top first cm ranged from 34 ± 46% to 66 ± 8.5% of the total abundance over 10 cm sediment depth. The PERMANOVA results indicated a significant effect of the interaction factor habitat by sediment depth ('HaxDe'; Main test, P(perm): 0.042; Table 5). Density differences were however restricted to the 1 - 2 cm layer (Pairwise test, clam beds - microbial mats P(MC): 0.02; microbial mats - non-seeps P(MC): 0.03) and the 5 - 10 cm layer (Pairwise test, clam beds - non-seeps P(MC): 0.04). Density differences ascribed to the type of habitat, location and interaction between both were not found.

Table 3. Synthetic table with meiofauna densities (ind./10cm²), nematode dominance (%), nematode densities (ind./10cm²), nematode biomass (µg dwt/10cm²), Hill's diversity indices (H_0, H_1, H_2, H_{inf}), the rarefaction diversity index EG(51), mean relative abundance of feeding types according to Wieser (1953) (1A: selective deposit feeders, 1B: non-selective deposit feeders, 2A: epigrowth feeders, 2B: predators/scavengers), and mean trophic diversity (Θ^{-1}). Data are presented as averages with indication of the standard deviation, per location, per habitat.

Location	Habitat	Meiofauna density (ind./10cm²)	Nematode dominance (%)	Nematode density (ind./10cm²)	Nematode biomass (µg dwt/10cm²)
500 - 600 m	Microbial mat	161.3 ± 96	96.5 ± 5	158 ± 100	10.2
	Clam bed	504.5 ± 20	80.0 ± 17	395 ± 87	53.5
	Non-seep	633.0 ± 52	85.5 ± 5	538 ± 19	29.9
700 - 800 m	Microbial mat	350.2 ± 113	95.9 ± 4	338 ± 123	4.2
	Clam bed	689.9 ± 200	90.0 ± 4	626 ± 207	44.4
	Non-seep	1236.9 ± 568	97.7 ± 0	1209 ± 557	37.8
800 - 900 m	Microbial mat	44.0	87.9	40	16.8
	Clam bed	920.5	94.3	869	55.9
	Non-seep	336.8 ± 40	92.1 ± 1	310 ± 33	20.6

H_0	H_1	H_2	H_{inf}	EG(51)	Feeding types				Trophic index Θ^{-1}
					1A	1B	2A	2B	
9.0 ± 4	2.8 ± 2	2.1 ± 1	1.6 ± 1	3.2 ± 1	0.9 ± 1	93.3 ± 9	5.7 ± 8	0.0	1.2 ± 0.2
27.0 ± 3	8.3 ± 2	5.4 ± 1	3.1 ± 0	8.0 ± 2	13.1 ± 8	59.5 ± 13	25.9 ± 7	1.6 ± 2	2.3 ± 0.5
54.0 ± 9	27.3 ± 7	19.9 ± 5	10.0 ± 3	15.5 ± 1	38.9 ± 4	33.1 ± 4	22.2 ± 4	5.8 ± 5	3.2 ± 0.4
22.5 ± 11	4.9 ± 1	2.9 ± 0	1.8 ± 0	5.6 ± 2	2.6 ± 0	91.1 ± 7	5.8 ± 6	0.5 ± 1	1.2 ± 0.2
34.0 ± 1	6.3 ± 2	2.9 ± 1	1.7 ± 0	7.4 ± 0	7.8 ± 1	79.9 ± 4	11.1 ± 5	1.2 ± 0	1.5 ± 0.1
48.0 ± 11	17.5 ± 2	9.7 ± 1	4.0 ± 1	12.9 ± 0	32.7 ± 3	40.7 ± 0	23.2 ± 1	3.5 ± 2	3.1 ± 0.1
21	9.0	5.5	3.3	9.6	10.2	74.5	13.5	1.8	1.7
22	4.5	2.4	1.6	6.1	10.9	83.8	4.6	0.7	1.4
73.0 ± 4	39.0 ± 8	26.6 ± 7	10.7 ± 3	15.7 ± 1	23.6 ± 0	34.9 ± 2	31.8 ± 4	9.7 ± 2	3.5 ± 0.1

Table 4. Main test results from univariate PERMANOVA and PERMDISP analyses testing for differences in meiofauna density and taxon richness, and nematode dominance, biomass and diveristy between different locations (Lo: 500 - 600m, 700 - 800 m, 800 - 900 m), habitats (Ha: non-seep, clam bed, microbial mat), and the interaction term (LoxHa). Significant results are indicated in bold. ([a]: $0.001 < p \leq 0.05$, [b]: $p \leq 0.001$)

Test	Factors		Meiofauna density	Meiofauna taxon richness	Nematode dominance	Nema bion
PERMANOVA	Location	df	2	2	2	2
		MS	226180	4	78	6
		Pseudo-F	4.15	0.57	1.50	0.08
	Habitat	df	2	2	2	2
		MS	520370	16	34	1253
		Pseudo-F	**9.56** [a]	2.53	0.65	**17.39** [a]
	LoxHa	df	4	4	4	-
		MS	130890	4	54	-
		Pseudo-F	2.40	0.66	1.05	-
	Residuals	df	7	7	7	4
		MS	54450	6	52	72
	Total	df	15	15	15	8
PERMDISP	Habitat	F	1.95			0.16
		df 1	2			2
		df 2	6			6

atode nass	Nematode diversity					
	H_0	H_1	H_2	H_{inf}	EG(51)	Θ^{-1}
	2	2	2	2	2	2
	89	71	50	12	24	0.13
	1.74	3.67	4.18	3.96	4.11	1.91
	2	2	2	2	2	2
	2417	869	415	66	323	5.28
	47.19 [b]	**44.93** [b]	**34.35** [b]	**22.66** [a]	**56.33** [b]	**76.37** [b]
	4	4	4	4	4	4
	170	68	41	8	22	0.21
	3.31	3.51	3.39	2.69	3.85	3.07
	7	7	7	7	7	7
	51	19	12	3	6	0.07
	15	15	15	15	15	15
	1.63	2.46	3.20	3.20	0.60	0.09
	2	2	2	2	2	2
	6	6	6	6	6	6

Figure 2. The average absolute nematode and meiofauna densities (individuals per 10cm^{-2}, horizontal bars and dots, respectively) with indication of the standard deviations for the three depth zones (500 - 600 m, 700 - 800 m and 800 - 900 m).

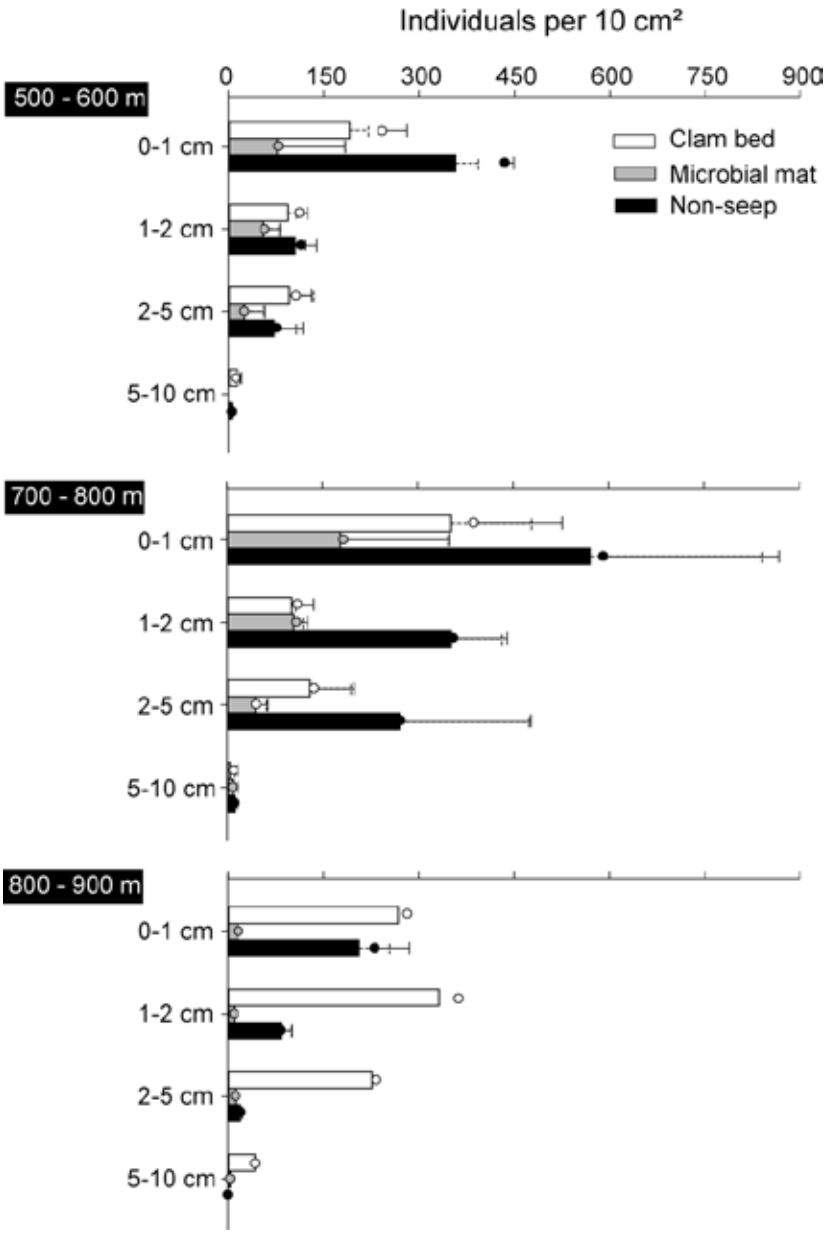


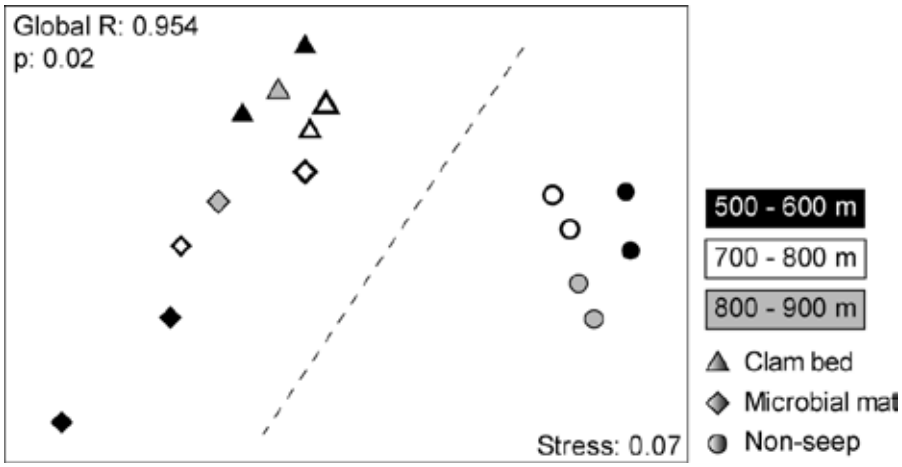
*Table 5. Main test results from multivariate PERMANOVA and PERMDISP analyses testing for differences in nematode density, and genus and trophic composition between different locations (Lo: 500 - 600m, 700 - 800 m, 800 - 900 m), habitats (Ha: non-seep, clam bed, microbial mat), sediment depths (De: 0 - 1 cm, 1 - 2 cm, 2 - 5 cm), replicates nested within habitats (Re(Ha): A, B), and interaction terms. Significant results are indicated in bold. The significance of the main effect of LoxRe(Ha) on the nematode genus composition is not discussed in the text because low replicate numbers did not allow to test for homogeneity of dispersions (PERMDISP). [a]: 0.001 < p ≤ 0.05, [b]: p ≤ 0.001, **: term has one or more empty cells)*

Test	Factors		Nematode density	Nematode genus composition	Nematode trophic composition
PERMANOVA	Location	df	2	2	2
		MS	40952	3912	366
		Pseudo-F	2.63	**3.05** [a]	2.15
	Habitat	df	2	2	2
		MS	59748	22794	8643
		Pseudo-F	5.77	**10.70** [a]	**19.56** [a]
	Depth	df	3	2	2
		MS	107590	2280	76
		Pseudo-F	**27.93** [b]	**3.30** [a]	0.53
	Re(Ha)	df	3	3	3
		MS	10530	2161	448
		Pseudo-F	1.49	**3.19** [b]	2.89
	LoxHa	df	4	4	4
		MS	37433	3262	594
		Pseudo-F	2.3	**2.54** [a]	3.49
	LoxDe	df	6	4	4
		MS	7711	853	68
		Pseudo-F	1.09	1.26	0.44
	HaxDe	df	6	4	4
		MS	14365	1176	298
		Pseudo-F	**3.67** [a]	**1.70** [a]	2.10
	LoxRe(Ha)**	df	4	4	4
		MS	15600	1283	170
		Pseudo-F	2.21	**1.89** [a]	1.10
	Re(Ha)xDe	df	9	6	6
		MS	3772	691	142
		Pseudo-F	0.53	1.02	0.91
	LoxHaxDe	df	11	8	8
		MS	3652	791	98
		Pseudo-F	0.52	1.17	0.63
	Residuals	df	9	8	8
		MS	7058	678	155
	Total	df	59	47	47
PERMDISP	Habitat	F	1.15	1.96	1.32
		df 1	2	2	2
		df 2	9	6	6
	Depth	F	3.56	3.10	
		df 1	3	2	
		df 2	8	6	
	Location	F		1.56	
		df 1		2	
		df 2		6	

Nematode assemblages

A total of 129 genera belonging to 31 families were identified. A PERMANOVA analysis of nematode genera revealed significant effects of the interaction of habitats by sediment depth ('HaxDe'), location by habitat ('LoxHa') and location by replicates nested in habitats ('LoxRe(Ha)') (Table 5). Due to limited replication of the different habitats at each of the three locations, the interaction factor 'LoxRe(Ha)' could not be properly interpreted. Pair-wise comparisons identified differences in nematode assemblages over the complete sediment profile between clam beds and microbials mat versus non-seep sites, for both the 500 - 600 m and 700 - 800 m location (P(MC) < 0.05). No significant differences were found between the nematode assemblage composition of clam beds and microbial mats, nor did the location and thus water depth have any effect on the nematode assemblages at the non-seep sites (P(MC) > 0.05). These results were visualized in an MDS plot (Fig. 3).

Figure 3. Non-metric multidimensional scaling (MDS) plot based on distance between centroids of nematode abundance data per cm sediment depth for the three different habitats at each depth zone. The global R value of the Two-Way crossed ANOSIM test for differences between habitat groups across all location groups indicates the strength of the difference between the seepage habitats and the non-seep habitats across the depth zones.



Results from the SIMPER analysis demonstrated that the similarity of nematode assemblages within seepage habitats at each location is more variable and lower than within non-seep sites (seepage sites: 42 - 69%, non-seep sites: 71 - 74%; Table 6). This nematode assemblage variability within as well as between seepage habitats, is partly created by highly variable

relative abundances of the dominant genera *Daptonema* and *Metadesmolaimus*. Both belonged to the top five most abundant genera in clam beds and microbial mats at all three water depths with relative abundances ranging from 7.2 - 85.2% (Table 6). Both genera are represented by only one species at the seep sites. Both the *Daptonema* sp. (male; L: 1294.4 ± 74.0 µm, a: 36.7 ± 2.8, b: 5.8 ± 0.3, c: 9.2 ± 0.7) and the *Metadesmolaimus* sp. (male; L: 761.2 ± 88.6 µm, a: 43.4 ± 7.6, b: 7.5 ± 1.0, c: 9.5 ± 2.0) are presently undescribed. These two species were also found at the three non-seep sites, with the dominant seepage *Daptonema* sp. representing 48 to 90% of all *Daptonema*'s found there, while only one and the same *Metadesmolaimus* sp. occurred at seep and non-seep sites. Other dominant genera occurring with > 5% mean relative abundance at a certain seepage site are *Leptolaimus*, *Morlaixia*, *Thalassomonhystera* and an undescribed genus spp. of the Chromadoridae family. Common dominant genera (> 3%) at all three non-seep sites were *Daptonema*, *Acantholaimus*, *Tricoma*, *Monhystrella* and *Halalaimus*. A total of 59 genera (50.4% of all genera, max. 18.8% of total density) were exclusively found at non-seep locations. Most of these genera were rare as only eight occurred with > 1% mean relative abundance at one of the three locations. At the seepage sites, 11 genera (15.7% of all genera, max. 11.5% of total density) occurred that were not found at the non-seep sites, with 3 genera being present with > 1% mean relative abundance in one of the habitats at a certain location (*Cephalochaetosoma*, *Metacylicolaimus*, gen. 1 spp.1 (Chromadoridae)). With 13 genera exclusively found in microbial mats and 15 genera exclusively found in clam beds, none of the habitat-specific seepage assemblages is a subset of the other, at any of the studied locations.

Table 6. Mean relative abundance of dominant (> 3%) genera per habitat, per location, over 5 cm sediment depth and results from the analysis of similarities and genera contributions (SIMPER) indicating percentages of (dis)similarities. Genera contributing to > 5% of the similarity within a habitat are marked with a star (*).

500 - 600 m	**Non-seep**	%	**Clam beds**	**%**	**Microbial mats**	%
	Leptolaimus *	9.08	*Daptonema* *	35.44	*Metadesmolaimus* *	85.20
	Daptonema *	9.02	*Linhomoeus* *	18.90	*Daptonema* *	8.63
	Molgolaimus *	8.06	*Leptolaimus* *	12.00	*Linhomoeus*	3.10
	Tricoma *	7.00	*Morlaixia* *	8.18	Rest	3.06
	Acantholaimus *	6.44	*Metadesmolaimus* *	7.23		
	Monhystrella	4.72	*Neochromadora*	3.14		
	Campylaimus	4.33	*Chromadorella*	3.09		
	Halalaimus	4.09	Rest	12.02		
	Sabatieria	3.88				
	Thalassomonhystera	3.88				
	Desmodora	3.85				
	Rest	35.66				
Non-seep	**Sim.**	**70.85**				
Clam beds	**Dissim.**	**75.09**	**Sim.**	**49.49**		
Microbial mats	**Dissim.**	**89.28**	**Dissim.**	**64.12**	**Sim.**	**49.97**

700 - 800 m	**Non-seep**	%	**Clam beds**	%	**Microbial mats**	%
	Daptonema *	24.51	*Daptonema* *	60.13	*Daptonema* *	51.43
	Acantholaimus *	11.53	*Metadesmolaimus* *	7.55	*Metadesmolaimus* *	23.39
	Leptolaimus *	9.56	*Intasia* *	4.00	*Thalassomonhystera*	6.92
	Elzalia *	7.07	*Chromadorina* *	3.07	Rest	18.26
	Halalaimus *	6.03	*Terschellingia* *	3.06		
	Tricoma	4.43	Rest	22.20		
	Microlaimus	3.90				
	Terschellingia	3.26				
	Monhystrella	3.10				
	Rest	26.60				
Non-seep	**Sim.**	**73.50**				
Clam beds	**Dissim.**	**59.01**	**Sim.**	**68.63**		
Microbial mats	**Dissim.**	**70.22**	**Dissim.**	**46.18**	**Sim.**	**41.86**

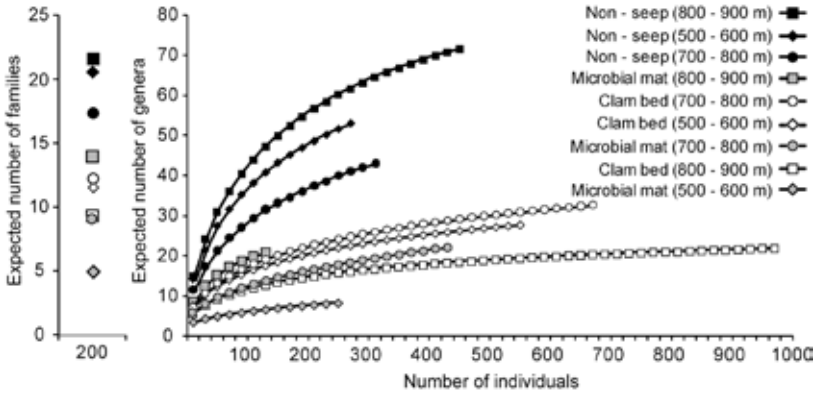
800 - 900 m	**Non-seep**	%	**Clam beds**	%	**Microbial mats**	%
	Daptonema *	10.01	*Daptonema*	70.38	*Metadesmolaimus*	31.67
	Acantholaimus	7.08	*Leptolaimus*	7.33	*Daptonema*	28.54
	Monhystrella	6.15	*Metadesmolaimus*	5.19	gen. 1 spp. 1 (Chromadoridae)	6.68
	Tricoma	6.14	*Anticoma*	4.78	*Morlaixia*	5.05
	Elzalia	4.29	Rest	12.31	*Metacylicolaimus*	4.80
	Microlaimus	4.26			*Promonhystera*	4.23
	Desmoscolex	3.74			*Leptolaimus*	3.26
	Dichromadora	3.69			Rest	15.77
	Actinonema	3.53				
	Halalaimus	3.14				
	Rest	47.98				
Non-seep	**Sim.**	**72.01**				
Clam beds	**Dissim.**	**75.09**	**Sim.**	**N/A**		
Microbial mats	**Dissim.**	**73.06**	**Dissim.**	**53.83**	**Sim.**	**N/A**

A multivariate PERMANOVA test based on feeding type relative abundances revealed a significant effect of the habitat type ('Ha'; Main test, P(perm): 0.023, Table 5). Significant differences in the composition of feeding groups occurred between microbial mats and clam beds versus non-seep sites (Pairwise test, P(MC): 0.006 and P(MC): 0.04, respectively), but not between microbial mats and clam beds (Pairwise test, P(MC): 0.26). The microbial mats and clam beds were characterized by a high dominance of non-selective deposit feeders (1B; relative abundances range from 59.5 ± 13% to 93.3 ± 8.9%; Table 3), while at the non-seep sites three feeding groups were common: the selective and non-selective deposit feeders and the epigrowth feeders (1A, 1B, 2A; relative abundances range from 22.2 ± 3.8% to 40.7 ± 0.1%; Table 3). Not any of the nematodes showed evidence of chemosynthetic symbionts based on light microscopy.

Nematode structural and trophic diversity

Both the structural (H_0, H_1, H_2, H_{inf}, EG(51)) and trophic (Θ^{-1}) diversity indices (Table 3), regardless of the emphasis given to relatively more or less abundant genera by the Hill's indices, were only significantly higher for the non-seep sites compared to both seepage habitats at all locations ('Ha'; Main tests, P(MC) $\leq$ 0.002; Table 4; Pairwise tests P(MC) $\leq$ 0.005). Genus and trophic type diversity did not differ between microbial mats and clam beds (Pairwise tests, P(MC) $\geq$ 0.06). The order of means of Hill's indices per habitat per location is reflected in the Expected number of Genera rarefaction curves (EG(n); Fig. 4) where means of replicates were plotted. The same trend was also found at the family level and visualized as EF(200) (Fig. 4). Although no significant difference was found due to a high variability, structural diversity corresponded to trophic diversity at the non-seep sites, with highest average index values at 800 - 900 m water depth, followed by the 500 - 600 m and 700 - 800 m sites (Table 3). Structural and functional diversity did not correspond at the seepage sites.

Figure 4. EF/G(n) rarefaction curves of the expected number of nematode families (EF) and genera (EG) for a given number of sampled individuals (n). Means of replicates were plotted for each type of habitat at every location.

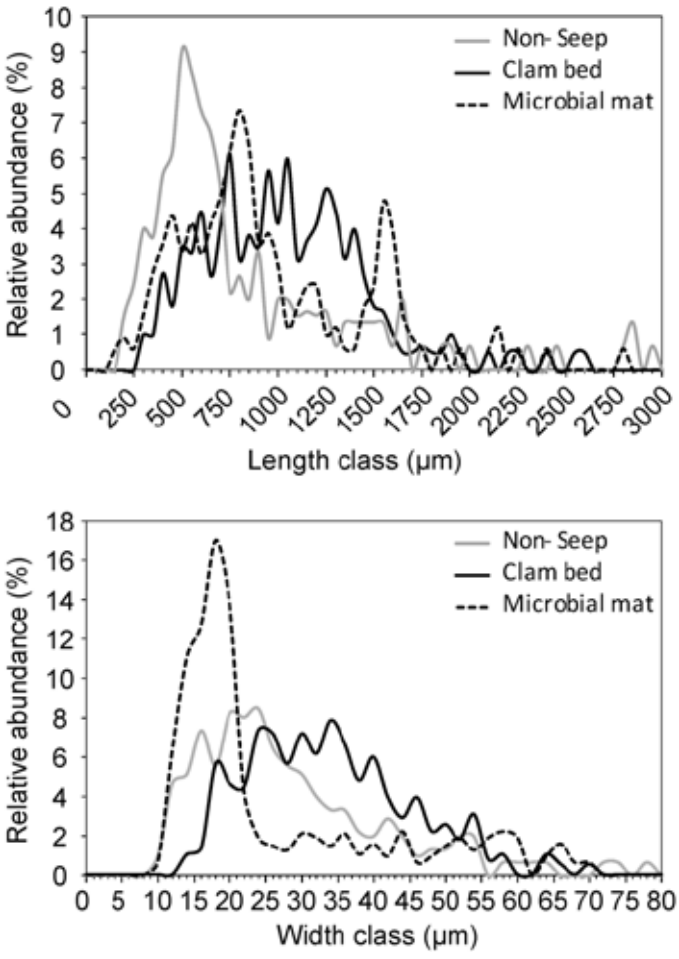


Nematode biomass and morphometry

It is legitimate to interpret the biomass and morphometry results of the first sediment centimeter as a proxy for the total community as the PERMANOVA results showed before that there was no difference in nematode assemblages with sediment depth within any of the habitats ('LoxHaxDe'; Main test, P(perm): 0.268; Table 5; Pairwise test for pairs of levels of 'De', within 'Lo' and within 'Ha', P(MC) ≥ 0.22). Generalisations about total biomass can however, not be made as densities in the top first centimeter are not representative for the complete sediment profile. Nevertheless, total nematode biomass was lowest in the top first centimeter of microbial mats (4.2 - 16.8 µg dry weight per 10 cm^{-2}), intermediate at the non-seep sites (20.6 - 37.8 µg dry weight per 10 cm^{-2}), and highest at the clam beds (44.4 - 55.9 µg dry weight per 10 cm^{-2}) (Table 3). Significant differences were only found between the microbial mats and clam beds ('Ha'; Main test, P(perm): 0.013; Table 4; Pairwise test, P(MC): 0.001). These total biomass values do not reflect the nematode densities alone but are clearly influenced by nematode individual biomasses (i.e. body size). Averaged over the three depths, highest biomass in the clam beds was linked to a fraction of nematodes with higher individual length (mean: 994 ± 395 µm) and width (mean: 31.7 ± 10.3 µm) compared to the non-seep nematodes (mean length: 707 ± 518 µm, mean width: 26.0 ± 12.2 µm; Fig. 5). Corresponding relative length frequency distributions differed significantly (χ^2 test, χ^2 = 122.8, df = 91, p = 0.015), but relative width frequency distributions did not (χ^2 test, χ^2 = 55.4, df = 50, p = 0.28). The clam bed biomass mainly increased due to the occurrence of nematodes with lengths from 1000 to 1500 µm and width from 30 to 55 µm represented by 35.8 ± 6.5% of the total clam bed nematode assemblages. This fraction of nematodes was mainly represented by *Daptonema* (mean: 93.9 ± 3.2%). Nematodes in the microbial mats were also relatively long

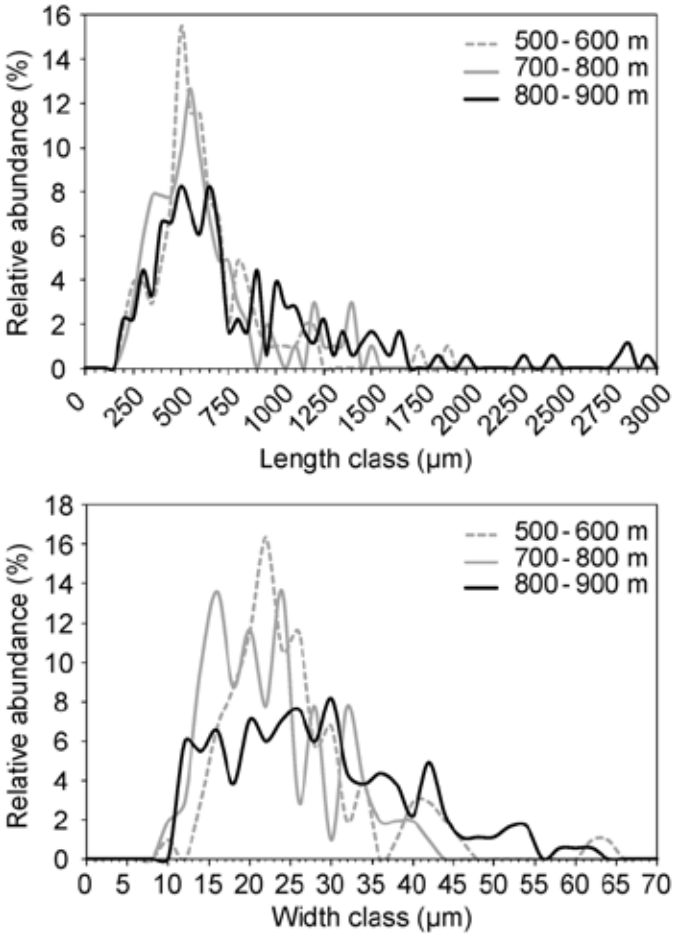
on average (mean: 1014 ± 1052 µm), but had the lowest mean individual width (mean: 23.6 ± 14.2 µm). Mean individual length and width in the microbial mats was however strongly influenced by the sample from 500 - 600 m water depth where *Metadesmolaimus* was most numerous (186 versus 161 and 64 individuals, respectively), and was much thinner (mean width: 16.2 ± 5.4 versus 28.3 ± 16 µm at 700 - 800 m and 33.6 ± 16 µm at 800 - 900 m).

Figure 5. Relative abundance (%) of nematode individuals in (A) Length geometric classes and (B) Width geometric classes, from the three habitat types: Non-seep, Clam bed and Microbial mat; based on the average of one replicate per location.



When we focus on the oxygen gradient at increasingly deeper non-seep locations it is clear that the nematode assemblage at the deepest site is characterized by a larger fraction of nematodes that are relatively longer and wider (only in one occasion significant: χ^2 test for nematode length, 800 - 900 m versus 700 - 800 m station, $\chi^2 = 102.1$, df = 50, p = 0.00002) (Fig. 6), and therefore induce a relatively higher total biomass compared to the two shallower sites (37.8 µg dry weight 10 cm^{-2} versus 29.9 and 20.6 µg dry weight 10 cm^{-2}; Table 3). While 75% of the nematodes at the 500 - 600 m and 700 - 800 m water depth have lengths smaller than 685 µm and 662 µm; and a smaller width than 28 µm and 27 µm, respectively, 75% of the nematodes at the deepest site have a smaller length than 1005 µm and smaller width than 36 µm.

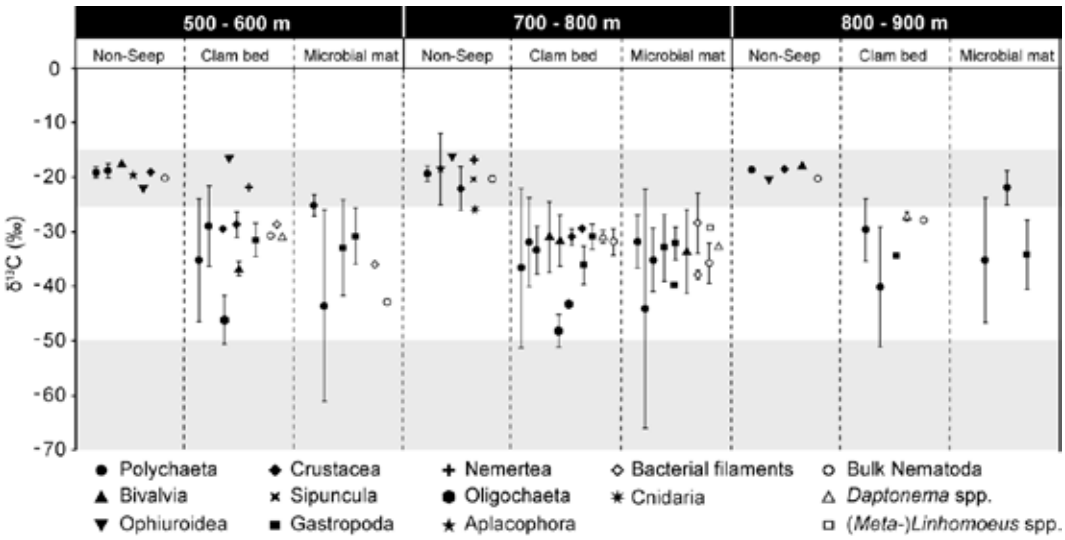
Figure 6. Relative abundance (%) of nematode individuals in (A) Length geometric classes and (B) Width geometric classes, from the non-seep sites at 500 - 600 m, 700 - 800 m and 800 - 900 m water depth, based on one replicate.



Faunal $\delta^{13}C$ values

Nematode and macrofauna individual $\delta^{13}C$ values exhibited clear distinctions based on the habitat type only at the 500 - 600 m and 700 - 800 m locations ('Ha'; Main tests, P(perm) ≤ 0.0007; Table 7). No individual taxon related patterns were found. PERMANOVA pairwise tests found that $\delta^{13}C$ values differed significantly between seepage habitats (clam bed and microbial mat) and non-seep sites (Pairwise tests, P(MC) ≤ 0.024), but not between the two seepage habitats (Pairwise tests, P(MC) ≥ 0.138). Because the PERMDISP analysis gave a significant difference in data deviation from the centroids among groups prior to the pairwise 'Ha' analysis for the 700 - 800 m site, it is unclear if the pairwise test results are based on data dispersion, location or most likely as seen in Fig. 7, a combination of both.

Figure 7. $\delta^{13}C$ (‰) isotope values (mean ± standard deviation where n ≥ 2, per taxon per sampling campaign) of bacterial filaments, nematodes and macrofaunal taxa collected at the different habitats (non-seep, clam bed, microbial mat) at all three locations (500 - 600 m, 700 - 800 m, 800 - 900 m). The grey area from -15‰ to -25‰ indicates the range of $\delta^{13}C$ values obtained for benthic consumers that mainly depend on phytoplanktonic carbon. The grey area below -50‰ indicates the assimilation of mainly methanotrophically derived carbon.



At the non-seep sites, all faunal $\delta^{13}C$ values averaged per higher taxon level and per sampling date, fell more or less well within the range of -15‰ to -25‰ (Fig. 7), indicating that the assimilated carbon in the nematodes and macrofauna was mainly of phytoplanktonic origin. Taxon mean $\delta^{13}C$ values at the non-seep locations ranged from -16.2 to -25.9‰, while mean

$\delta^{13}C$ values for non-seep nematodes and macrofauna over all locations were -20.12 ± 0.46‰ and -19.45 ± 3.25‰, respectively. The maximum MDC percentage for single non-seep taxa measurements ranged from 0% to 13%, with positive values measured in ophiuroids, an aplacophoran, an amphipod, and a cnidarian.

*Table 7. Main test results from univariate PERMANOVA and PERMDISP analyses testing for differences in $\delta^{13}C$ values between habitats (Ha: non-seep, clam bed, microbial mat), taxa (Ta: Polychaeta, Bivalvia, Ophiuroidea, Gastropoda, Crustacea, Sipuncula, Nemertea, Oligochaeta, Aplacophora, Cnidaria, Nematodea, bacterial filaments), and the interaction term (HaxTa); separately tested for each location (500 - 600m, 700 - 800 m, 800 - 900 m). Significant results are indicated in bold. ([a]: 0.001 < p ≤ 0.05, [b]: p ≤ 0.001, **: Term has one or more empty cells)*

Test	Factors		500 - 600m	700 - 800 m	800 - 900 m
PERMANOVA	Habitat	df	2	2	2
		MS	899	1420	82
		Pseudo-F	**10.54** [b]	**15.48** [b]	1.38
	Taxa	df	2	2	2
		MS	18	7	92
		Pseudo-F	0.21	0.08	1.54
	HaxTa**	df	3	2	-
		MS	14	74	-
		Pseudo-F	0.16	0.80	-
	Residuals	df	100	202	33
		MS	85	92	60
	Total	df	107	208	37
PERMDISP	Habitat	F	4.3	**45.1** [a]	
		df 1	2	2	
		df 2	5	4	

In the clam beds across the seepage sites, nematode $\delta^{13}C$ values differed significantly between the two shallowest and the deepest location ('Lo'; Pairwise test, P(perm) ≤ 0.001), with nematodes from the 800 - 900 m location depending significantly less on MDC than the nematodes from the 500 - 600 m and 700 - 800 m sites (MDC maxima: 18.6 ± 1.5% versus 25.4 ± 0.7% and 26.1 ± 5.4%, respectively; Pairwise test, P(perm) ≤ 0.0004). The polychaete and gastropod data showed no pattern as such ('Lo', Main test, P(perm) ≥ 0.55). Most taxa from the seepage sites showed a wide range of carbon isotopic signatures (Fig. 7), reflecting varying nutritional sources across taxa within a site and across sites within a taxon. Mean $\delta^{13}C$ values for higher taxa ranged from -16.2‰ to -48.1‰, indicating that 0% to maximum 59 ± 11% of their carbon was derived from methane. The majority of the seepage nematodes and

macrofauna had mean $\delta^{13}C$ values between -27‰ and -40‰ (i.e. minimum 0% to maximum 46% MDC). $\delta^{13}C$ values of polychaetes, gastropods, and bivalves at several seepage sites, however, also showed overlap with carbon isotope signatures indicative for phytoplankton derived carbon ($\delta^{13}C$ > -25‰; Fig. 7), emphasizing a mixed diet observed when combining species into higher taxa. Measurements on a single ophiuroid and nemertean specimen that were occasionally found in a clam bed at 500 - 600 m water depth had fairly high $\delta^{13}C$ values, indicating the incorporation of mainly phytoplankton derived carbon ($\delta^{13}C$: -16.4‰ and -21.7‰, respectively). Isotopic signatures of large, filamentous bacteria forming visible white and orange mats at the two shallowest seepage sites, were indicative for chemosynthetic carbon fixation via sulfide oxidation, with averages ranging from -28.4 ± 5.6‰ to -37.8 ± 0.9‰.

DISCUSSION

Metazoan meiofauna in Hydrate Ridge sediments

Sommer et al. (2007) presents the only study to date on metazoan meiofaunal standing stocks at HR. The surprisingly high dominance of rotifers at clam beds, microbial mats and control sediments often exceeded nematode dominance (rotifers: 4 - 85%, nematodes: 13 - 90 %), and differs from our results (rotifers: 0%, nematodes: 80 - 98%; Appendix A). Despite the discrepancy in occurrence of rotifers, the mean nematode density (individuals per 10 cm^{-2}) reported by Sommer et al. (2007) for each habitat type corresponds well with the means observed in this study, irrespective of water depth (microbial mats: 162.7 versus 206.4, clam beds: 546.5 versus 578.6, 921.7 versus 685.5). In decreasing order of relative abundance, copepods and nauplii, and polychaetes followed in dominance and nematodes showed distinct surface (0 - 2 cm) maxima in both studies. According to Giere (2009), the colonization of anoxic, sulphidic sediments at HR by the rotifer species is amazing, considering their preference for well-oxygenated sediments. We therefore suggest that based on (i) the more or less equal vertical distribution of rotifers in the sediment in each sediment core, (ii) the non-significantly differing densities in each sediment layer between all studied seep habitats and non-seep sites, and (iii) the occurrence of only one species, provisionally identified as the cosmopolitan fresh and brackish water species *Lecane closterocerca* (Shmarda 1859) (Dr. H. Segers, personal communication) in the study of Sommer et al. (2003, 2007), in addition to (iv) the general lack or very low abundances of rotifers in deep-sea marine environments with avoidance of hypoxic conditions (Neira et al. 2001), that the occurrence of this rotifer as described by Sommer et al. (2003, 2007) might possibly result from contamination. Rotifera were identified before as the dominant taxon in fresh tap water, reaching up to 1500 individuals per m^3 of water (Schellart 1988; Jeurissen and Vermaercke 1990; Funch et al. 1996).

Seep contribution to nematode community heterogeneity

While seep locations with similar habitat types as found at HR exist, geographic heterogeneity manifests through variations in water depth, intensity of fluid flow, long-term stability, availability of nutrients, the variety of substrates present, CH_4 and H_2S concentrations and the presence or absence of an OMZ affecting both the seep and its surrounding sediments (Cordes et al. 2010). The combination of all these factors may have contributed to some extent to the establishment of a unique nematode community at HR. Average genus richness at HR seep habitats was relatively high, while evenness corresponded well with the average Shannon diversity index and Pielou's evenness for known other deep-sea seeps (Vanreusel et al. 2010a). This reflects the fact that the HR nematode assemblages exhibit a typical trend of high dominance by one or two species representing 50 - 90% of the total community (Vanreusel et al. 2010a). At HR, *Daptonema* and *Metadesmolaimus* together represented 43 - 94% of the total community, whereas a significantly higher nematode diversity was observed, as expected, in the non-seep sediments at all three sampled water depths, with similar trends observed at the genus and family level. Since on average slightly lower nematode individuals were identified in the non-seep compared to the seep samples (414 ± 121 versus 551 ± 263, respectively), diversity in the non-seep reference sites might even be underestimated. Seep and non-seep sediments shared only 50% of their nematode genera, which is similar to the result found for the macrofauna (Levin et al. 2010a) and emphasizes the significance of the HR seeps to regional diversity.

At the smallest scale, varying fluid fluxes and availability of chemicals largely determine the habitat heterogeneity at spatial scales relevant for microbiota but also for the symbiont-bearing mega- and macrofauna such as tubeworms, mussels and clams (MacDonald et al. 1989; Cordes et al. 2010). The higher structural complexity created by the presence of habitat-forming clam beds at HR resulted in higher diversity of the associated macrofauna (Sahling et al. 2002; Levin et al. 2003). Our data, in contrast, indicated that nematode communities at HR do not consistently profit from clam bed increased habitat heterogeneity in terms of diversity. Beside the pumping and oxygenating effect on the sediment down to 5 cm depth, which may both influence the macro- and meiofauna, *Calyptogena* clams induce an increased habitat complexity to the sediment surface where they visually form extended beds. Macrofaunal epizoonts living on the shells (gastropods, actinians, dorvilleids and scale polychaetes; Levin 2005) or crawling and swimming macrofaunal species may additionally be using the shells as e.g. refuge or settling and feeding grounds. Additionally, in contrast to the macrofauna at HR where the microbial mat fauna differed from the clam bed fauna (Sahling et al. 2002) or appeared largely to be a stress-tolerant subset of the clam bed fauna (Levin et al. 2010a), the nematode communities could not be distinguished based on genus composition. The within-

habitat heterogeneity (at the scale of tens of centimeters to a meter between tube cores) was on average as high as the between-habitat heterogeneity for clam beds and microbial mats, indicating a low genus turnover rate between the different seepage habitat types. The turnover both within and between the seep habitats mainly was manifested as a shift in dominance at the nematode genus level, with the genera *Daptonema* and *Metadesmolaimus* as key players.

Our study reports the first dominant *Metadesmolaimus* seep-associated species, whereas Shirayama and Ohta (1990) also reported two *Daptonema* species dominating with 33% relative abundance underneath *Calyptogena soyoae* clam beds at the Hatsushima seep site, Sagami Bay (Northwest Pacific Ocean). There is however no information available to verify whether one of them is the same *Daptonema* species that co-dominates with *Metadesmolaimus* at the HR seep sites. Nevertheless, what distinguishes both the HR and Hatsushima seep from other seeps for which nematode data are available is their occurrence within low oxygen realms. In the Sagami Bay intense mineralization in the water column results in a relatively stable oxygen-deficient layer reaching minimum values of ca. 55 - 60 µmol L^{-1} at 1200 - 1400 m water depth (Glud et al. 2009), while the seep is found at 1170 m water depth (Shirayama and Ohta 1990). At both seep locations (HR and Sagami bay) the dominant seep-associated *Daptonema* and some other less dominant genera (relative abundances $\geq 3\%$) also occurred as (sub)dominant genera in the surrounding non-seep sediments. These findings favor the idea that oxygen stress creates a pool of species that are tolerant to low-oxygen levels; which may colonize the seeps and further adapt to sulphidic conditions to increase their local success. This is in contrast to what has been found previously at deep-sea seeps located in well-oxygenated waters, where generally the dominant nematode species were not or only rarely encountered in background sediments. Instead, these dominant species were morphologically similar to species that are common at intertidal habitats over a broad geographical range (e.g. *Halomonhystera disjuncta*, *Terschellingia longicaudata* and *Sabatieria mortenseni*; reviewed in Vanreusel et al. 2010a). It seems feasible that these species tolerate high sulfide and methane concentrations in shallow marine environments where high loads of organic matter can rapidly deplete the sediment of oxygen and induce biogenic production of reduced elements, while they become largely outcompeted in relatively food-poor, well-oxygenated deep-sea sediments. Deep-sea cold seeps located near shore on margins are exceptions that could be accessed by drifts of loose seaweed (e.g. *Fucus* sp.), as assumed for the Håkon Mosby Mud Volcano (Van Gaever et al. 2009c).

Metazoan standing stock (densities and biomass) along the regular slope generally decreases with water depth and surface primary productivity (Rex et al. 2006). It is expected that both endosymbiont-carrying and heterotrophic metazoans at deep-sea seeps profit from the

excess supply of autochthonous organic carbon in addition to the carbon input through the water column, which may lead to elevated densities and/or biomass compared to adjacent non-seep sediments. Nematode densities at seeps vary from around 10 to 11,100 ind. 10 cm^{-2} (reviewed in Vanreusel et al. 2010a), and often exceed densities in the background sediments. Densities at the HR seepage habitats varied from 40 to 869 ind. 10 cm^{-2} and only exceeded densities in the surrounding margin sediments at 800 - 900 m water depth. It should, however, be noted that total nematode abundances at the non-seep reference sites could be underestimated due to the use of a 42 µm mesh sieve compared to the 32 µm mesh sieve that was generally used at the seep sites (Leduc et al. 2010). The clam beds that are characterized by sulfide concentrations mostly below 2 mM in the top five centimeters of the sediment generally attained higher meiofaunal and nematode densities than the bacterial mats where sulfide concentrations can reach over 28 mM (Sahling et al. 2002; Valentine et al. 2005; Sommer et al. 2007). The fact that the proportional vertical distribution of the nematodes in the HR sediments did not differ between clam beds and microbial mats, illustrates however that sulfide concentrations might not necessarily hamper nematode densities. Oxygen penetration in microbial mats at HR is less than a few millimeters (Knittel et al. 2003), but similar to sulfide, it did not seem to influence the nematode abundances and distribution in the sediment. Neither did the overall quantity of organic carbon that was estimated to be almost twice the amount in microbial mats compared to clam beds (Valentine et al. 2005). Therefore, we expect that it is rather the combination of the transient nature of seepage, seep intensity and interactions with the habitat-structuring and/or associated benthic taxa that determine nematode success.

In terms of biomass, nematode assemblages at the seep habitats at HR confirm the suggested proportionally increased biomass compared to non-seep assemblages. A higher biomass, induced by a fraction of nematodes with relatively higher length and width, indicates at least a fraction (mainly represented by *Daptonema*) of the nematodes at the clam beds may profit from the high local chemosynthetic production, despite other factors interfering in determining density. Van Gaever et al. (2009a) suggested the same for *S. mortenseni*, the dominant nematode genus present in mussel and clam habitats at the REGAB pockmark (Gulf of Guinea, South-East Atlantic). The same seems true for the nematodes in the HR microbial mats, except for one microbial mat at 500 - 600 m water depth, where *Metadesmolaimus* dominated, representing 93% of the total meiofaunal community, whereas in other measured microbial mat samples its relative abundance was at most 52%, and a maximum of 5% in clam bed samples. As the microbial mat and clam bed nematode assemblages were indistinguishable but entailed a high within- and between-habitat variability, by selecting only one replicate per location for body measurements, we might have seen genus-dependant patterns rather than habitat-type induced differences in geomorphometric characteristics.

Our results however, might illustrate that where the survival of other nematode genera is hampered, *Metadesmolaimus* might have an advantage form elongated body-length and therefore higher surface area-to-volume ratio; a characteristic that was suggested to be an adaptation to low oxygen partial pressure, epidermal uptake of DOM and a high mobility for nematodes living in deeper suboxic or anoxic conditions (Jensen 1986, 1987b; Schiemer et al. 1990; Soetaert et al. 2002).

OMZ effects on nematode communities

Most diversity research within OMZs has focused on Foraminifera, macrofauna, or megafauna. These groups are characterized by a low number of taxa, low species richness and diversity, and high dominance of a few species when compared with more oxygenated surrounding habitats (Levin et al. 2001; Levin 2003, Gooday et al. 2009, 2010). The only study on metazoan meiofauna diversity within and outside an OMZ reports a considerable drop in number of taxa with decreasing oxygen concentrations and increasing organic carbon due to the absence of taxa other than nematodes (Veit-Köhler et al. 2009). The non-seep study locations at HR however, are all situated close to the OMZ core. Therefore the range of bottom-water oxygen concentrations at the different sampled water depths (± 0.7 - 0.2 O_2 ml l^{-1}) might have been too small to observe any clear elimination effect on meiofaunal taxa that are less tolerant to oxygen stress. Comparing the rarefaction index for nematode genus diversity (EG(51)) between the OMZ sediments at HR and average global slope sediments (Vanreusel et al. 2010b) indicates that nematode genus diversity within the OMZ at HR is nevertheless relatively low (14.7 ± 1.6 versus 19.0). On a regional scale, average nematode diversity was highest at the deepest non-seep location with lowest oxygen concentrations (800 - 900 m water depth; Table 1). Although diversity usually increases with increasing water depth along the upper continental slope (Rex et al. 1983), this was not the case at HR, where average diversity was lowest at intermediate water depth. Carbon and nitrogen concentrations in the sediment increase with increasing water depth at the non-seep margin locations off Oregon (500m: 0.9% C, 0.1%N; 800m: 2.14% C, 0.28% N (Levin et al. 2010a); 770m: 1.99% C, 0.25% N (Valentine et al. 2005), but also do not affect nematode diversity. However, as structural (based on the nematode community composition) and functional trophic (based on feeding types according to Wieser 1953) diversity show the same trend with water depth, we assume that rather than quantity, the composition or the quality of the sedimentary organic matter influenced nematode diversity.

The nematode assemblages from the non-seep margin sediments within the OMZ consist of several genera that are known for their widespread distribution in the deep sea (*Acantholaimus*, *Desmodora*, *Desmoscolex* and *Halalaimus*) (Vanreusel et al. 2010b).

Together with the genera *Daptonema, Microlaimus, Sabatieria* and *Thalassomonhystera*, which each contribute more than 2.5% average relative abundance in slope habitats worldwide (Vanreusel et al. 2010b), these genera contributed each more than 3% to the similarity between replicates of at least one of the sampled Cascadian non-seep margin locations. The most remarkable difference in relative abundances between the assemblages found in this study and the averages for genera found in slope sediments worldwide is the higher relative abundance of *Daptonema* and the lower relative abundances of *Sabatieria* and *Thalassomonhystera* in our study (*Daptonema*: 14.8 ± 8.6%, *Sabatieria*: 2.1 ± 1.7%, *Thalassomonhystera*: 2.3 ± 1.6, versus 5.51%, 8.71% and 9.65%, respectively). Whether this variability is the result of the oxygen minima found on the Cascadian margin or other variables, is a question that cannot be answered at this time. Including nearby margin sediments outside the OMZ will be necessary to find out if the OMZ sediments favor the success of nematode species that are tolerant (colonists) or adapted (endemics) to low oxygen levels. Moreover, it will also reveal which species additionally (colonists) or alternatively (endemics) occur at seepage sites due to lack of tolerance of most other metazoan life or a competitively successful adaptation, respectively. In the study of Levin et al. (2010), where the macrofaunal assemblages were studied at seep and non-seep sediments at depths of 800 m and 500 m on the Oregon and Californian margins, only four species were confined to the OMZ setting outside of seeps. This suggests that the relatively weak NE Pacific OMZ contributes only minimally to regional macrofaunal diversity (Gooday et al. 2010). However, as nematodes have a different life style, being smaller, less mobile organisms with no pelagic larval stage, it is reasonable to speculate that OMZs have isolated hypoxia-tolerant nematode species more easily e.g. as a result of expanding or contracted OMZs, resulting from shifts in global temperatures. Once isolated, speciation may be further promoted (Rogers 2000).

Levin et al. (2010a) hypothesized that seep-related heterogeneity would be homogenized where bottom-water oxygen concentrations are lowest. A comparison of macrofaunal seep and non-seep assemblages from HR and Eel River did not support this hypothesis (Levin et al. 2010a). Nor do our nematode assemblages along a water depth and bottom-water oxygen gradient at HR. The dissimilarity between assemblages in surrounding non-seep sediments and seepage habitats at HR decreased with decreasing O_2 concentrations from 500 - 600 m to 700 - 800 m water depth (75.09 and 89.28 versus 59.01 and 70.22, respectively), but increased again towards 800 - 900 m water depth (75.09 and 73.06), where the O_2 concentrations were lowest.

Metazoan meiofauna and nematode densities often reach maximum values at lowest oxygen concentrations within OMZs (Levin 2003). The data from the present study and the study by Veit-Köhler et al. (2009) illustrate that this is not always true. Lowest nematode densities at HR were reached at the deepest, least oxygenated location (886 - 887 m) while highest

densities occurred at the intermediate depth (797m) (Table 1). Macrobenthos densities in margin sediments at HR followed the same pattern, with annelids dominating the macrobenthos at each depth with relative abundances ranging from 67.5 to 76.2% (Levin et al. 2010a). In the OMZ of the Arabian Sea, nematode densities were also positively correlated with total macrobenthos and annelid abundances, in particular with the dominant tube-building family Spionidae (Cook et al. 2000). Possibly the effect of different annelid or other macrofaunal species that burrow, bioturbate and/or bio-irrigate deep-sea sediments might induce higher nematode survival rates in a similar way as has been shown for different tube-building polychaetes in intertidal and shallow subtidal sediments (Reise 1981; Tita et al. 2000; Pinto et al. 2006; Braeckman et al. 2010).

Reduced body size and flattened tests, which both lead to an increase in the surface area-to-volume ratio, are typical features of some OMZ metazoan macrofauna and hypoxia-tolerant foraminiferal species, respectively (Levin 2003; Gooday et al. 2010). We speculated that a body elongation and subsequent increase in surface-volume ratio might also emerge in the upper sediment layer along the investigated depth and oxygen depletion gradient, as was suggested before for nematodes living in and being adapted to deeper suboxic or anoxic conditions (Jensen 1986, 1987b; Schiemer et al. 1990; Soetaert et al. 2002). The minimum bottom-water oxygen concentrations at 800 - 900 m water depth seemed to have this body elongation effect on part of the community. However as it co-occurred with an increase in body width, the resulting increased biomass seems more likely the result of higher concentrations of food in the sediment.

Importance of methane-derived carbon for macrofaunal and nematode communities

Cold seeps are isolated areas that provide opportunities for higher energy exploitation in the deep sea that is often considered food limited. At HR, methane released from decomposing gas hydrates induces microbial autotrophic processes which provide the benthic food web with additional organic carbon. Based on $^{14}CO_2$ fixation experiments, a 24-fold increase in autochthonous production of organic carbon was measured at the southern HR seep sites (700 - 800 m) in comparison to 5.7 mg C m^{-2} d^{-1} at control sites (Sommer et al. 2002). The presence of distinct communities of macrofaunal and nematode species that feed on this chemosynthetic derived carbon is evidenced by both the results from the macrofaunal and nematode community composition and ^{13}C stable isotope analyses in this present study and the study of Levin and Michener (2002) and Levin and Mendoza (2007). At the non-seep margin locations average $\delta^{13}C$ values were -20.12 ± 0.46‰ for nematodes and -19.45 ± 3.25‰ for macrofauna, the latter corresponding well with the results from Levin and Michener

(2002) who found -20.78 ± 0.92‰ at the southern non-seep Oregon margin site (770 m). These signatures result from the incorporation of mainly phytoplankton derived carbon. While Levin and Michener (2002) found distinct macrofaunal $\delta^{13}C$ values for microbial mats (-43.80 ± 3.63‰), clam beds (-33.38 ± 2.17‰) and non-seep sediments (-20.78 ± 0.92‰), our study only found seep versus non-seep related differences for all analyzed benthic taxa. High variance between and within the seep-associated higher taxa was responsible for this, and indicates species consume a variety of food sources. There was no greater incorporation of carbon derived from methane or sulfide oxidation in microbial mats, as found by Levin and Michener (2002), who sampled HR 7 years earlier. Mean macrofaunal and nematode $\delta^{13}C$ values ranged from -16.2‰ to -48.1‰, which corresponds to 0% to maximum 59 ± 11% MDC. A more detailed interpretation of the stable isotope signatures is however complex due to the variety of potential food sources. On the one hand there is MDC which can be obtained through endosymbiotic methanotrophs, or through direct or indirect consumption of free-living anaerobic (archaea) or aerobic methane-oxidizing bacteria, or sulfide-oxidizing or sulfate-reducing bacteria, which also can take up MDC after it passes into the dissolved inorganic carbon (DIC) pool (Thurber et al. 2010). On the other hand at HR there is an unlimited DOC-supply with a $\delta^{13}C$ isotopic composition of -36.5‰ that is assumed to contribute up to 60% of the carbon in seep sediments (Valentine et al. 2005). This seeping DOC may be taken up directly through uptake of sediment particles to which DOC is absorbed (Sposito et al. 1999; Kennedy et al. 2002) or indirectly after being accumulated in intermediate consumers such as heterotrophic sulfate-reducing prokaryotes (Knittel et al. 2003). A third potential carbon source is phytoplankton-derived carbon, which can also be taken up directly or indirectly. A mixture of several of these carbon sources may yield faunal $\delta^{13}C$ signatures that were observed for the majority of the macrofauna and nematodes (i.e. between -27 and -40‰), and which are indistinguishable from those associated with form I Rubisco sulfide oxidation (i.e. -27 to -35‰; Robinson and Cavanaugh 1995; Cavanaugh and Robinson 1996). Moreover, a combined consumption of sulfide oxidizers having form I and II Rubisco (i.e. -27 to -35‰ and -9 to -16‰, respectively; Robinson and Cavanaugh 1995; Cavanaugh and Robinson 1996) may yield tissues with phytoplankton-like $\delta^{13}C$ signatures (Levin and Michener 2002).

Hitherto none of the seep nematode species in the deep sea have shown evidence of symbionts. Instead, most nematodes from seeps are classified as deposit feeders, based on their small buccal cavity and the absence of teeth (Vanreusel et al. 2010a). At HR, especially the non-selective deposit feeders (group 1B according to Wieser 1953) dominated at all seepage sites with relative abundances ranging from 59.5 ± 13‰ to 93.3 ± 8.9‰. Combined analyses of fatty acids and stable isotopic signatures of the non-selective deposit feeding nematode *H. disjuncta* from the Nordic margin indicated that this species proliferates on the highly abundant sulfide-oxidizing bacteria, and is therefore trophically adapted to conditions

within the Håkon Mosby Mud Volcano bacterial mats (Van Gaever et al. 2009b). Besides evidence of a mixed diet on autochthonous and allochtonous organic carbon, $\delta^{13}C$ signatures of seep-associated nematodes at HR reveal a significantly lower MDC % or potentially higher portion of phytoplankton derived carbon in the nematodes' diet at the deepest site, compared to the shallower sites. This might reflect an increased availability of phytodetritus at that depth as suggested by the highest concentrations of carbon in the sediment at the deepest non-seep site. To elucidate however, the potential occurrence of chemoautotrophic endosymbionts, trophic level, carbon and nitrogen sources and fixation pathways in the nematodes at HR, it is thus highly recommended to use dual ($\delta^{15}N$ and $\delta^{13}C$) stable isotope measurements preferably on nematodes and bacteria, as well as fatty acid-specific $\delta^{13}C$ analysis.

CONCLUSION

Knowledge of trends in nematode diversity and assemblages at cold seeps and OMZs has steadily grown during the last decade. Our efforts reveal that both seep and OMZ sources of margin heterogeneity generate distinct and unique nematode assemblages, although they share certain environmental and faunal characteristics with seeps and OMZs worldwide. The OMZ homogenizes the habitat-related heterogeneity within the HR seep, but the homogenizing effect on nematode assemblages from seep and non-seep sediments did not increase along the relatively narrow bathymetric and bottom-water oxygen gradient. Relative to non-seep assemblages the HR nematode seep assemblages exhibited low diversity with dominance of only one or two genera (in this case *Daptonema* and *Metadesmolaimus*), higher average nematode individual biomass, and $\delta^{13}C$ evidence for strong dependence on chemosynthesis-derived carbon. These attributes are shared with deep-sea seeps worldwide. This study is the first however, to reveal that nematode communities at seeps under the influence of an OMZ may differ from those at deep-sea seeps in well-oxygenated water. In the latter the dominant genera are rarely encountered in the oxygenated background sediments and presumably originate from sulfide-rich, shallow areas. In contrast an OMZ may host a potential source of infaunal species adapted to low oxygen levels, which can then colonize and further adapt to the prevailing reducing seep conditions. Future taxonomic work that combines morphological and molecular characterizations of nematode species from HR sediments, adjacent oxygenated slope sediments, shallow-water sediments and nearby cold seeps is however, required to resolve the degree of endemicity at the HR seep and OMZ.

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ADDENDUM

Appendix A. Mean relative abundances of meiofauna taxa per sediment cm layer and over 0 - 10 cm depth with standard deviation, per habitat for each location.

500 - 600 m	**Clam bed**					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
Amphipoda	0.17	0.13	0.0	0	0.13	0.18
Bivalvia	0	0	0	0	0	0
Cnidaria	0.04	0	0	0	0	0
Copepoda	19.93	8.27	5.63	5.94	11.15	13.99
Cumacea	0.06	0	0.17	0	0.06	0.09
Halacarida	1.04	0.90	0.52	0	0.67	0.94
Isopoda	0.06	0.13	0	0	0.06	0.09
Kinorhyncha	0.06	0	0	0	0.03	0.04
Nauplii	1.70	4.71	4.27	0	2.97	3.60
Nematoda	**73.31**	**81.45**	**85.58**	**84.97**	**80.01**	**16.98**
Oligochaeta	0.11	0.91	2.04	8.68	0	0
Ostracoda	0.41	0.13	0.07	0.41	0.10	0.13
Polychaeta	3.06	3.37	1.72	0	4.80	2.13
Tanaidacea	0.06	0	0	0	0.03	0.04

700 - 800 m	**Clam bed**					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
Amphipoda	0.09	0.15	0	0	0.08	0.05
Aplacophora	0	0	0	0	0	0
Bivalvia	0	0	0	0	0	0
Cnidaria	0	0	0	0	0	0
Copepoda	6.62	2.67	1.79	3.13	4.97	0.74
Cumacea	0	0	0	0	0	0
Gastropoda	0	0	0	0	0	0
Gastrotricha	0	0	0	0	0	0
Halacarida	0	0	0	0	0	0
Isopoda	0.05	0	0	0	0.03	0.04
Kinorhyncha	0.11	0.15	0	0	0.09	0.13
Nauplii	2.82	1.53	0.68	0	2.14	1.13
Nematoda	**87.37**	**92.75**	**95.65**	**96.88**	**90.02**	**3.91**
Oligochaeta	0.08	0.40	0.17	0	0.16	0.17
Ostracoda	0.26	0	0.17	0	0.19	0.21
Polychaeta	2.61	2.35	1.53	0	2.32	1.69
Tanaidacea	0	0	0	0	0	0

800 - 900 m	**Clam bed**					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
Aplacophora	0	0	0	0	0	
Bivalvia	0	0.09	0	0	0.03	
Cnidaria	0	0	0	0	0	
Copepoda	0.88	0.94	1.06	0	0.91	
Echinodermata	0	0	0	0	0	
Gastrotricha	0	0	0	0	0	
Halacarida	0	0	0	0	0	
Isopoda	0	0.17	0	0	0.07	
Kinorhyncha	0	0	0	0	0	
Nauplii	1.10	4.03	1.46	0	2.29	
Nematoda	**95.03**	**91.60**	**96.81**	**98.55**	**94.30**	
Oligochaeta	0	0	0	0	0	
Ostracoda	0	0	0	0	0	
Polychaeta	2.98	3.17	0.66	1.45	2.40	
Turbellaria	0	0	0	0	0	

	Bacterial mat						Non-seep					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
	0	0	0	0	0	0	0.02	0	0	0	0.01	0.02
	0	0	0	0	0	0	0.56	0.21	0.26	0	0.46	0.32
	0	0	0	0	0	0	0.0	0	0	0	0	0
	1.17	0.28	0	0	1.17	1.65	7.39	2.90	2.10	2.50	5.90	2.35
	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0.14	2.50	0.03	0.05
	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0.03	0.10	0	0	0.04	0.06
	0.20	0	0	0	0.07	0.10	8.00	2.42	0.73	0	6.02	1.63
	97.06	**98.35**	**99.39**	**0**	**96.53**	**4.52**	**82.08**	**92.34**	**94.49**	**88.89**	**85.49**	**4.56**
	0	0	0	0	0	0	0.08	0.07	0.07	0	0.07	0.04
	0.20	0	0	0	0.17	0.24	0.25	0.12	0.07	0	0.21	0
	1.37	1.38	0.61	0	2.07	2.73	1.53	1.71	2.08	6.11	1.68	0.22
	0	0	0	0	0	0	0.06	0.12	0.07	0	0.08	0.01

	Bacterial mat						Non-seep					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
	0.26	0	0	0	0.08	0.05	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0.02	0	0	0.01
	0	0	0	0	0	0	0.02	0.02	0	0	0.01	0.02
	0	0	0	0	0	0	0	0.02	0	0	0	0.01
	0.92	1.06	1.08	0	0.96	0.75	2.35	0.24	0.16	3.16	1.26	0.17
	0	0	0.26	0	0.06	0.08	0	0	0.05	0	0.01	0.01
	0	0	0	0	0.02	0.03	0	0	0	0	0	0
	0	0	0	1.02	0.06	0.08	0.04	0.02	0	0	0.02	0.03
	0	0	0.26	0	0.06	0.08	0.02	0.02	0	0.63	0.02	0.02
	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0.04	0.07	0.02	0	0.04	0.06
	0.23	0.36	0.26	0	0.29	0.41	0.22	0.05	0.11	0.63	0.14	0.04
	93.61	**95.80**	**97.88**	**98.98**	**95.91**	**4.14**	**96.50**	**98.86**	**98.77**	**91.44**	**97.67**	**0.19**
	0.47	0	0	0	0.11	0.16	0	0	0.10	0.63	0.03	0.02
	0	0	0	0	0	0	0.02	0	0	0	0.01	0.01
	4.51	2.55	0.26	0	2.39	2.47	0.80	0.72	0.78	3.50	0.78	0.17
	0	0.12	0	0	0.06	0.08	0	0	0	0	0	0

	Bacterial mat						Non-seep					
	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.	0 - 1	1 - 2	2 - 5	5 - 10	Total	St.dev.
	0	0	0	0	0		0.04	0	0	0	0.02	0.03
	0	0	0	0	0		0.28	0	0.36	0	0.22	0.12
	0	0	0	0	0.04		0	0	0	0	0	0
	3.26	0	0	0	6.63		9.18	0.84	2.96	0	6.66	0.81
	0	0	0	0	0		0.04	0	0	0	0.02	0.03
	0	0	0	0	0		0.10	0	0	0	0.06	0.04
	0	0	0	0	0.39		0	0	0	0	0	0
	0	0	0	0	0		0	0	0	0	0	0
	0	0	0	0	0		0.29	0	0	0	0.21	0.24
	0	0	1.45	0	0.21		0.46	0	0.36	0	0.34	0.10
	89.1	**94.6**	**92.8**	**95.2**	**87.9**		**89.27**	**98.76**	**95.29**	**100**	**92.06**	**0.98**
	0	0	0	0	2.28		0	0	0.36	0	0.02	0.03
	0	0	0	0	0.36		0.17	0	0.31	0	0.13	0.07
	7.61	5.36	5.8	4.76	2.17		0.18	0.31	0.36	0	0.23	0.11
	0	0	0	0	0		0	0.10	0	0	0.02	0.03

6

General discussion, conclusions and future challenges

There are many arguments to assume that nematodes are an important component of the deep-sea benthos. They are by far the most abundant metazoans, and they constitute a considerable portion of the metazoan biomass (Gage and Tyler 1992). Nematodes may also play a role in deep-sea food webs as link between basal resources and higher trophic levels. In shallow water it was demonstrated that they alter the sea bed by adding mucus, by creating burrows, and by tube-building (Thistle et al. 1995 and references therein). These processes have been shown to stimulate microbial activity and/or decomposition processes (Moens et al. 2005b and references therein). Deep-sea nematodes may have similar effects. This magnanimous assumption already yielded the hypothesis that in benthic deep-sea ecosystems, a higher nematode diversity might imply a higher functional diversity and as such promote ecosystem processes (Danovaro et al. 2008b). Despite the potential importance of nematodes, our understanding of their ecology in the deep sea is still in the "pattern-recognition stage" as was nicely expressed by Thistle et al. (1995).

In the following, the insights in deep-sea nematode ecology provided by this doctoral study are situated, integrated and discussed in the context of the current state of knowledge and future work.

ADVANCES IN DEEP-SEA NEMATODE FEEDING ECOLOGY: PAST AND PRESENT KNOWLEDGE

Building on shallow water environment facts and techniques

Marine free-living nematodes are capable of consuming bacteria, microalgae, detritus, fungi, protozoan and metazoan prey, as well as taking up dissolved organic matter (DOM). Empirical evidence of specialized or selective feeding on specific food resources was found for several estuarine nematode species after they were successfully brought in lab cultures (e.g. Jensen 1987a; Moens and Vincx 1997; Moens et al. 1999; Moens et al. 2000; Hamels et al. 2001). These experiments confirmed that feeding habits are linked to mouth morphology as originally proposed by Wieser (1953), who was the first to introduce a feeding type classification (1A: selective and 1B: non-selective deposit-feeders, 2A: epistrate-feeders and 2B: omnivore-carnivores). At the same time, however, it became clear that similar mouth structures may be used for very different feeding behaviors, and different mouth morphologies may serve very similar feeding behaviors. In accordance with experimental findings and observations, alternative feeding type classifications and modifications were proposed (Romeyn and Bouwman 1983; Jensen 1987a; Moens and Vincx 1997). It remains, however, dubious to classify a species or genus in a feeding group since several species and genera have shown

to feed in a facultative or obligate manner on certain food sources or exhibit trophic level switching during development (i.e. life-history omnivory; Yeates 1987a, b; Hellwig-Armonies et al. 1991; Moens et al. 2006 and references therein). Hence, it seems that much more data are needed on the feeding habits of individual nematode species before an ecologically valid grouping of nematodes into feeding categories can be attempted (Ólafsson et al. 1999). Overall, the previous mentioned lab experiments indicated that phenomena like temporal and species-specific feeding plasticity, species interactions, prey-density dependent predation rates, and the small fraction of nematode species successfully cultured in the lab, hinder proper estimates of the quantitative importance of potential food sources in the nutrition of coastal or estuarine nematode communities by means of lab experiments. No deep-sea nematode was ever brought successfully into culture (except for *Halomonhystera disjuncta* from the Håkon Mosby Mud Volcano which survived in culture for 4 months; Van Campenhout, personal comments). As such, no comparative genus- or species-specific observations on food preferences, selectivity, feeding rates or plasticity could ever be made. Instead deep-sea nematode ecologists tend to rely on the Wieser classification (Wieser 1953) to look for ecological differences between study locations, ecosystems or habitats. Suggestions, for example, that state that the dominance of epistrate feeders is a functional adaptation to the short-term event of fresh food supply to the deep seafloor (Vanhove et al. 1995, 1999, 2004; Sebastian et al. 2007) were, however, not empirically supported.

The methodological advance of tracer techniques provided the possibility to isotopically label autochthonous microphytobenthos (with ^{14}C- or ^{13}C-bicarbonate) and bacteria (with ^{3}H-thymidine, ^{3}H-leucine, ^{14}C-amino acids, ^{13}C-bicarbonate, ^{13}C-acetate, ^{13}C-toluene, ^{13}C-glucose) or add prelabeled food particles in controlled field or microcosm set-ups and follow the uptake through different, relatively undisturbed and natural, benthic compartments. The downside of this approach is the considerable biomass requirements to perform C isotope analyses compared to the relatively small individual biomass of nematodes. This often hindered analysis beyond nematode community levels or on less abundant genera or species. Nevertheless, these techniques enabled rate estimates of bacterivory and algal grazing and allowed the inclusion of nematodes in foodweb models that describe the exchange of matter among different compartments within an ecosystem (e.g. van Oevelen et al. 2006b, c, 2011).

Isotope tracer studies in coastal and intertidal estuarine sediments enabled successful tracing of algal and bacterial carbon into free-living nematodes. In general, nematodes showed a rapid reaction. They became enriched after 0.5 to 7 h, and ^{13}C or ^{14}C assimilation increased over time, until day 3 to 18 (Montagna 1984; Middelburg et al. 2000; Moens et al. 2002; van Oevelen et al. 2006a). In studies where both microphytobenthos or diatoms and bacteria were labeled in situ and in a microcosm experiments, nematode grazing on microphytobenthos and diatoms (with highest biomass) exceeded that on bacteria (Montagna 1984; Montagna and

Yoon 1991; Sundbäck et al. 1996). In the studies of Sundbäck et al. (1996) and van Oevelen et al. (2006a, b), where the contribution of bacterial carbon to the requirements of intertidal meio- and macrobenthos was quantified, bacteria appeared to be of minor importance to both (8% to 10% and 11% on average, respectively). Since the added bacterial tracer may appear in grazers by other pathways than grazing, e.g. adsorption or absorption, active transport or uptake by enteric or epicuticular bacteria (e.g. Montagna and Bauer 1988; Ólafsson et al. 1999 and references therein), the above mentioned contribution of bacteria to the nematodes' diet may even be overestimated. On the other hand, both microphytobenthos and phytoplankton carbon entered all heterotrophic components in proportion to heterotrophic biomass distribution (bacteria > macrofauna > meiofauna; Widbom and Frithsen 1995; Middelburg et al. 2000; or among nematode species; Moens et al. 2002).

In most deep-sea sediments, benthic consumers depend directly or indirectly on the sedimentation of phytodetritus, which typically occurs as a seasonal event. Deep-sea environments that are subject to such seasonal phenomena are generally considered to be food-poor and subject to periods of severe starvation. Exceptions are locations where mass deposits of organic matter occur (e.g. OMZ, whale fall event) or where chemoautotrophs thrive on seafloor effluxes of chemical energy (e.g. cold seeps, pockmarks, hydrothermal vents, mud volcanoes) and form an oasis of basal resources for a selected group of higher taxon consumers. Although the composition of the diet of nematodes at the Hydrate Ridge seep locations remains dubious, natural $\delta^{13}C$ stable isotope results evidenced that nematodes depend on chemosynthetic based resources (**Chapter 5**). With the focus on photosynthetic driven ecosystems, several tracer experiments have tried to quantify the importance of phytodetritus in the diet of different benthic compartments through the addition of ^{13}C-prelabeled algae. Bacteria, macrofauna and protozoa generally responded with a rapid (within 35 hours to 8 days) and considerable uptake of fresh phytodetritus (Blair et al. 1996; Levin et al. 1997, 1999; Moodley et al. 2000, 2002; Witte et al. 2003a, b; Aberle and Witte 2003; Nomaki et al. 2005; Aspetsberger et al. 2007; Woulds et al. 2007; Gontikaki et al. 2011a). The short-term uptake (traced between 35 hours to 23 days) by nematodes, however, was always absent (Nomaki et al. 2005; Sweetman and Witte 2008; Ingels et al. 2010b) or limited, occasionally after a time delay of 2 to 3 weeks (Moodley et al. 2002; Ingels et al. 2010a; Witte et al. 2003b; Moens et al. 2007; Gontikaki et al. 2011a). This lack of, or minute, uptake of phytodetritus by nematodes, together with arguments based on digestive constraints (Jumars et al. 1990), natural nematode and sediment $\delta^{15}N$ values (Iken et al. 2001), and density correlations between bacteria and nematodes (Vanreusel et al. 1995a; Hoste et al. 2007) arose speculations that bacteria instead may contribute significantly to the diet of deep-sea nematodes. This was tested in an onboard experiment in which the indigenous bacterial communities from the 1280 m HAUSGARTEN station were ^{13}C-labeled by different

organic and inorganic carbon sources (**Chapter 2**). The isotope dynamics of the nematodes were evaluated by an isotope tracer model to quantify the contribution of bacterial carbon to the nematodes' diet. From the relatively low nematode $\delta^{13}C$ values and the lack of a decent model fit we could deduce that nematodes did not depend on bacterial carbon for their metabolism over the observed time span of 7 days. The minimal absolute uptake (I) (mg ^{13}C ind^{-1} d^{-1}) measured in the nematodes only accounted for 0.1% to 5.1% of their theoretical daily metabolic requirements. These results are in accordance with Baguley et al. (2008), who conducted a 24-h term, in vitro experiment in which aerobic heterotrophic bacteria from the top first centimeter of deep-sea sediments from the Gulf of Mexico were labeled with tritiated thymidine (^{3}HTdr). In the tracer study performed by Ingels et al. (2010b), Southern Ocean deep-sea nematodes were significantly labeled over time (at day 1, 7, and 14) in the treatment where prelabeled bacteria were added. This label incorporation was, however, very low and nematode contribution to carbon mineralization was estimated to account for < 1%. The combined colonisation/tracer experiment performed in situ at the 2500 m HAUSGARTEN station additionally showed a lack of nematode feeding on both ^{13}C-prelabeled bacteria over a time span of 10 days (**Chapter 3**). Our experimental findings obtained in the Arctic deep sea were later qualitatively confirmed by field observations of fatty acid compositions of deep-sea nematodes from the Southern Ocean (**Chapter 4**). Bacterial fatty acids were generally of minor importance (max. 5.7%). Therefore, substantial proof excludes bacterivory as an answer to the time lag in detritus uptake observed by Witte et al. (2003a), Moens et al. (2007) and Ingels et al. (2010a) in different pulse-chase experiments. Neither are the feeding group classification of Wieser (1953) and the Jensen (1987a) interpretation, both assuming that a large fraction of the deep-sea nematode community is feeding selectively on bacteria, empirically supported. Instead, the deep-sea nematode fatty acid compositions and dual stable isotope ratios reinforce the importance of phytodetrital material as basic food resource (**Chapter 4**). Whether deep-sea nematodes feed directly or indirectly on phytodetritus is thoroughly discussed based on fatty acid compositions, in **Chapter 4**. Dual stable isotope ratios of both deep-sea nematodes and phytoplankton in the water column may further elucidate nematodes' trophic position. The latter data were, however, not determined from the ANDEEP-SYSTCO locations where the benthic samples were taken. We therefore rely on literature resources to try to further elucidate the trophic link. **Chapter 4** contains a summarized version of this discussion and focuses mainly on the latitudinal trend of the nematode $\delta^{13}C$ and $\delta^{15}N$ values. What follows is an extended version in which the cause of the latitudinal gradient is better explained and where speculations on the trophic position of nematodes are thoroughly argued. This extended discussion will be included in a paper solely dealing with the dual stable isotope analysis of both nematodes and copepods of the ANDEEP-SYSTCO locations (Veit-Köhler et al. in preparation).

Elaborated discussion on the dual stable isotope results along the ANDEEP-SYSTCO transect

$\delta^{13}C$ signatures of biogenic POM are controlled by biological fractionation during photosynthesis, and are negatively correlated with the aqueous CO_2 concentrations ($[CO_2(aq)]$) because of increased utilization of ^{13}C when CO_2 is in short supply. This was shown for several areas around the world (Zhang et al. 2011 and references therein), including the Southern Ocean (e.g. Fischer 1991; Kennedy and Robertson 1995; Lourey et al. 2004), and Antarctic seas (Rau et al. 1991a). The Southern Ocean has a meridional pattern of increasing surface water $[CO_2(aq)]$ and hence decreasing $\delta^{13}C_{CO2(aq)}$ with latitude (Gruber et al. 1999). Therefore, $\delta^{13}C$ in sea surface water POM correlates positively with temperature (SST) (Fontugne and Duplessy 1981; Rau et al. 1991a; Dehairs et al. 1997; Lara et al. 2010). Dehairs et al. (1997) formulated a regression equation based on measurements in the Atlantic section of the Southern Ocean ($\delta^{13}C_{POM}$ = 0.52 SST - 28.32). According to this equation, $\delta^{13}C_{POM}$ data at the ANDEEP-SYSTCO stations would be around -25.5‰ at the Polar Front, -27.4‰ at the south Polar Front, -29.3‰ at central Weddell Sea, -29.2‰ at Maud Rise, and -29.2‰ at the Lazarev Sea. This indicates differences in isotope values between surface water POM and the deep-sea nematodes ranging from +1.5‰ at Maud Rise to +5.0‰ at the south Polar Front. Depending on the potential increase in $\delta^{13}C_{POM}$ with water depth due to degradation and selective respiration of ^{12}C (Rau et al. 1991b), these isotopic shifts indicate that nematodes could be primary, but as well higher level consumers of fresh phytodetritus. Nevertheless, this remains a vague and rough speculation since other factors than $[CO_2(aq)]$ may have an important influence on $\delta^{13}C_{POC}$, which makes predictions based on sea surface temperatures alone hazardous. Examples of factors that may influence carbon isotopic compositions of photosynthesizing organisms are the species composition and related physiological differences (e.g. cell size, membrane permeability, type of carboxylating enzyme and growth rate), irradiance and day length, and zooplankton-related processes (Dehairs et al. 1997 and references therein; Zhang et al. 2011 and references therein).

A similar positive correlation between $\delta^{15}N_{POC}$ and SST (Lara et al. 2010) and inverse proportional relationship between $[NO_3^-]$ and $\delta^{15}N_{POC}$ or $\delta^{15}NO_3^-$ in surface water and upper sediments was found in the Atlantic, Pacific and Indian part of the Southern Ocean (Altabet and Francois 1994, 2001; Sigman et al. 1999; Robinson et al. 2004; Lara et al. 2010). Also here, this relation is not a function of nitrate concentration but of the fraction of unutilized substrate. Preferential incorporation of ^{14}N into phytoplankton biomass progressively enriches the nitrate in surface water with ^{15}N, resulting in a parallel increase in $\delta^{15}N$ of the newly formed biomass (Sigman et al. 1999 and references therein; Lara et al. 2010). $\delta^{15}NO_3^-$ in surface seawater gradually decreased from 8.9‰ to 6.3‰ between 47.51°S (just above the Polar Front) and

59.20°S at 115°E in the East Indian Ocean (Sigman et al. 1999). Since the Polar Front in the East Indian Ocean is situated at the same latitude as in the Atlantic Ocean along the Prime Meridian (ca. 49°S) a cautious interpretation could be made when comparing the surface water $\delta^{15}NO_3^-$ values with benthic nematode data. This indicates an enrichment of around 4‰ to 2‰ in the nematodes relative to surface water $\delta^{15}NO_3^-$ for the Polar Front and central Weddell Sea stations, respectively. Since aquatic consumers are on average 2.3‰ - 3.4‰ enriched in ^{15}N relative to their food source, the estimated isotopic shift between deep-sea nematodes and surface water NO_3^- suggests nematodes may feed on planktonic material as primary or secondary consumers.

Nematodes from the Lazarev Sea station, located closest to the continental shelf, are the exception to the general trend of enriched isotope values towards the Polar Front. Both $\delta^{13}C$ and $\delta^{15}N$ values are higher than expected according to the observed north-south gradient formed by the other stations. This likely reflects a dependence on pelagic phytoplankton released from light limitation as the ice edge retreats whether or not, and potentially temporarily, including ice algae released from melting sea-ice. Southern Ocean phytoplankton communities detected in spring and summer grow slowly, in light-limited conditions and in the presence of high [CO_2(aq)] which promotes strong carbon fractionation with typical light $\delta^{13}C$ values (< -30‰) as a result (e.g. Wada et al. 1987; Fischer et al. 1991; Rau et al. 1991a; Nyssen et al. 2002). Since nematode $\delta^{13}C$ values in our study are about 4‰ to 5‰ heavier, the contribution of sedimenting ice algae and/or krill fecal pellet strings, which typically have $\delta^{13}C$ values of around -20‰ to -25‰ upon the initial break-up of winter-ice around Antarctica (Wada et al. 1987; Bathmann et al. 1991; Fischer et al. 1991; Frazer 1996) is very likely. This idea is supported by findings at Kapp Norvegia (71° 07′ S, 12° 12′ W), close to the ANDEEP-SYSTCO Lazarev Sea sampling station. Here, $\delta^{13}C$ measurements on sedimented organic carbon revealed a temporal shift within one austral spring/summer period from -24.2‰ to -29.7‰ that could be attributed to a change of the organic matter source, related to the retreating sea-ice (Bathmann et al. 1991). An indication for the contribution of ice-algae in the diet of nematodes at the LS station was also found in their fatty acid composition that deviated from the other ANDEEP-SYSTCO stations (**Chapter 4**). Surface $\delta^{15}N$ values of POM in or closely associated with sea ice in the Weddell Sea, which corresponded to surface $\delta^{13}C_{POM}$ values of -20‰ to -25‰, ranged from 3‰ to 6‰ (Rau et al. 1991a). This corresponds to an isotopic shift of minimum 2.6‰ to maximum 7.2‰ with the nematodes sampled at the Lazarev Sea station in our study (8.6‰ - 10.2‰), indicating they might be first or up to third level consumers of sedimented POM. The potential enrichment in $\delta^{15}N_{POM}$ with depth, due to a selective loss of ^{14}N during respiration and degradation of POM (e.g. DeNiro and Epstein 1978; Wada et al. 1987), favors the idea that nematodes feed directly or as second level consumers on the sedimented POM. As well does the significant fraction of krill fecal strings and pellets in Weddell Sea POC fluxes at depth greater than 250 m (Nöthig and von Bodungen 1989;

Bathmann et al. 1991), taking into account that they are typically enriched in ^{15}N relative to food sources (by 2.2‰ in Altabet and Small 1990). On the other hand, since summer detritus pulses consist of large and fast sinking particulates, including intact aggregated diatom cells (Altabet et al. 1991; Bathmann et al. 1991), as was also evidenced by the green fluffy layer on top of the sediment at the revisited south Polar Front station along the ANDEEP-SYSTCO transect (Veit-Köhler et al. 2011), intermediate trophic levels cannot be excluded.

In conclusion, both fatty acid compositions and stable isotope values of nematodes from the Southern Ocean deep sea evidenced the trophic link between surface water primary production and deep-sea nematodes (**Chapter 4**). Low nematode fatty acid concentrations additionally suggest that deep-sea nematodes feed year-round on more degraded or constantly available food sources (e.g. fecal pellets, foraminiferans), supplemented by selective feeding on high quality food (phytodetritus) in circumstances of excess fresh labile detritus. This finding agrees with the suggested presence of an accumulated sediment food bank that buffers the benthic ecosystem from the seasonal variability of the water column, as proposed for the Antarctic shelf (500 - 1000 m water depth, Mincks et al. 2005; Smith et al. 2006). Nematode fatty acid compositions further indicate bacteria are of minor importance in their diet. At the same time the tracer experiments performed with Arctic deep-sea sediments evidenced no short-term feeding response on newly available patches of phytodetritus, nor did they evidence significant feeding on bacteria (**Chapter 2 and 3**). These results incite us to speculate on (1) slower respiration and growth rates than initially estimated based on organisms' biomass and water temperature (de Bovée and Labat 1993; Mahaut et al. 1995), and (2) the potential importance of DOM or other intermediate consumers that bridge the link between phytodetritus and nematodes. If one of these speculations holds true, deep-sea nematodes might be less food limited than is generally expected.

Deep-sea nematode rates of metabolism

It is expected that deep-sea animals possess cold-adapted, as well as pressure-adapted, biochemical systems. In the view of trophic ecology of the deep sea, there is likely to be selection for biochemical adaptations that establish the appropriate rates of metabolic activity (Somero 1992). Respiratory rates of pelagic fishes, crustaceans and cephalopods, for example, have been shown to decrease rapidly with increasing minimal depth of occurrence, mainly as a result of selection for reduced locomotory activity due to reduced light and distances over which predators and prey interact (e.g. Childress 1975, Sullivan and Somero 1980, Torres and Somero 1988, Seibel et al. 1997; Childress et al. 1990). Benthic invertebrates on the contrary, have relatively modest reductions in metabolic rate with increasing depth (Childress and Mickel 1985). Instead, reductions appear to reflect the

influence of temperature and body mass (Childress 1975; Childress and Mickel 1985). Broadly speaking, higher temperatures lead to higher metabolic rates within a species and smaller animals generally have higher mass-specific metabolic rates than larger ones (Drazen and Seibel 2007 and references therein). So far, however, in situ respiration rate measurements of isolated nematodes were technically not feasible. Linear regressions relating deep-sea nematode respiration to body weight and temperature were obtained from studies on shallow water nematodes and occasionally on measurements of Shirayama (1992) on depressurized deep-sea nematodes (de Bovée and Labat 1993 and references therein; Mahaut et al. 1995). Both the effect of the different methods used for the measurements (e.g. Cartesian divers or related techniques, polarographic oxygen electrodes) and decompression in the study of Shirayama (1992) are, however, unknown and therefore unaccounted for. The use of these regressions may induce estimated metabolism rates that are higher than actually present and underestimate the contribution of bacterial or phytodetrital carbon in nematodes' diets in tracer experiments. Additionally, actual lower metabolic rates would explain the generally slower response in short-term enrichment and colonisation experiments in deep sea compared to shallow, coastal areas (**Chapter 2** and **3**). With the urge of understanding the relationship between sediment community structure and seafloor ecological processes, there is a clear need for respiration measurements on deep-sea nematodes. The use of rapid and accurate methods, preferably under pressurized conditions, will allow the study of nematode metabolic allometry and determining their functional role in benthic metabolism.

Potentially underestimated resources

Alternatively or additionally to the slower metabolic rates assumed above, nematodes may be feeding on resources that are less limiting and generally not taken into account in food web models, since empirical data are scarce. In a recent carbon-flow modeling study for a sub-arctic benthic community, for example, it was estimated that 99% of the nematode carbon requirements were fulfilled by (unspecified) refractory detritus (Gontikaki et al. 2011b). Although the DOC stock was taken into account as a resource for bacteria, it was not considered as a potential food source for nematodes. Neither were the benthic protists.

Dissolved organic matter

Measurements on chlorophyll a (chl-*a*) and chloroplastic pigment equivalents (CPE) are widely used as tracers for the sedimentation of phytodetritus. However, they do not discriminate between the labile and the refractory components of detritus (Pfannkuche 1993). Although no universally accepted methodology of assessing the labile fraction of sedimentary organic matter yet exists, concentrations of carbohydrates (C), lipids (L) and proteins (P), and P:C or

C:L ratios have been used as indicators for quantity and quality, respectively (Danovaro et al. 1993; Tselepides et al. 2000 and references therein). In the deep Eastern Mediterranean, for example, spatial and vertical distribution of meiofauna appeared to be controlled by the food quality of the sediment (expressed as percentage of labile organic matter versus the total organic matter), rather than by its quantity (Danovaro et al. 1995; Danovaro et al. 2000). In slope sediments on the Peru margin, nematode abundance was positively correlated with both food input (CPE concentrations) and quality (concentrations of labile organic compounds) (Neira et al. 2001). These labile compounds occur partly in the pool of pore water dissolved organic matter (DOM) which originates from particulate organic matter (POM) remineralization (microbial hydrolysis and fermentation) (Otto and Blazer 1998 and references therein).

More than 80% of all sediment organic matter accumulates on continental margins (Berner 1982). This trend can reflect many possible factors. Besides high rates of organic production, rapid burial, low rates of degradation or mineralization, dominance of fine-grained sediments increases the surface area onto which OM can adsorb (Milliman 1994 and references therein). This adsorption of OM to mineral surfaces stabilizes the component molecules, slowing remineralization rates by up to five orders of magnitude (Keil et al. 1994 and references therein). Sorptive protection can therefore account for the enigmatic preservation of intrinsically labile molecules such as amino acids and simple sugars in marine deposits and links the preservation of organic carbon in marine sediments to the deposition of mineral surfaces (Dafner and Wangersky 2002). Resuspension of sediments enables desorption and releases DOM for remineralization (Keil et al. 1994).

DOM is mainly utilized by heterotrophic bacteria and eventually oxidized to dissolved inorganic carbon ($\sum CO_2$) through terminal metabolism (respiration) (Papadimitriou et al. 2002 and references therein). At the same time, both labile and refractory components of bacterial cells are released in the surrounding water as DOM through a variety of biological processes, including direct release, viral lysis and grazing (Ogawa et al. 2001 and references therein, Gruber et al. 2006). The importance of DOM in the nutrition of higher deep-sea taxa, however, is still speculative (reviewed in Gage 2003). This is also the case for marine free-living nematodes. Several studies provided experimental evidence for uptake of various DOM compounds (e.g. glucose, acetate) by nematodes brought in culture (Rothstein and Mayoh 1966; Chia and Warwick 1969; Rothstein 1970; Lopez et at. 1979; Montagna 1984; Riemann et al. 1990). So did the in vitro experiment performed at the HAUSGARTEN site (**Chapter 2**). Suggestions for carbon sources based on natural $\delta^{13}C$ values of both nematodes and pore-water DOC in cold seep sediments at the Hydrate Ridge also included DOC (**Chapter 5**). Acetate is a product of microbial fermentation processes, which together with glucose can be used for amino acid synthesis in some nematodes

(*Ditylenchus triformis*, *Meloidogyne incognita*, *Caenorhabditis briggsae*, *Aphelenchoides rutgersi*; summarized in Nicholas 1984). This may have interesting implications for trophic transfer (Moens et al. 2006). Since nematodes can use acetate for *de novo* synthesis of polyunsaturated fatty acids (PUFA) (Rothstein and Gotz 1968; Rothstein 1970; Bolla 1980), nematodes could represent an alternative to algae-derived PUFA that are essential for higher trophic levels (Phillips 1984). The fatty acid compositions of deep-sea nematodes from the ANDEEP-SYSTCO stations (**Chapter 4**) support this latter suggestion. From the in vitro experiment performed at the HAUSGARTEN site, it was however clear that nematodes did not accumulate the DOM compounds over the time span of seven days (**Chapter 2**). Whether the DOM substances were immediately respired or just passed the gut without accumulation was unclear. Therefore, further experimentation with axenic cultivations or incubations of nematodes is needed to elucidate the significance of DOM to the nutrition of nematodes. The use of stable isotope-labeled substrates in combination with fatty acid specific isotope analysis may further provide information on the nematodes' ability for *de novo* biosynthesis.

An explanation that is worth considering with regard to the lack or delay and limited response of nematodes in pulse-chase experiments or natural phytodetritus inputs, is the competition with smaller size groups for the available resources, including DOM. Danovaro et al. (2000) hypothesized the competition between bacteria and meiofauna based on the field observation of meiofaunal densities that showed seasonal changes where bacteria accounted for less than 60% of the total benthic biomass and not where bacteria accounted for 90% or more of the total benthic biomass. Gooday et al. (1996) on the other hand, attributed the rapid response of foraminiferans compared to meiofauna to 1) the higher growth rates of protozoans, 2) the rapid physiological re-awakening of some foraminiferans as a response to sudden food influxes, and 3) the highly mobile network of granuloreticulate pseudopodia which constitutes an efficient system for trapping, gathering and accumulating food particles. Overall, the combination of slower metabolic rates and DOM uptake seems feasible especially since deposit feeders that selectively feed on organic matter particles may ingest sediment with higher bacterial abundances as compared with bulk sediment (Plante and Jumars 1993; Andresen and Kristensen 2002). Therefore if nematodes were feeding according to their assumed metabolic rates, this likely would have resulted in considerable labeling of the nematodes in our tracer experiment, which it did not (**Chapter 2**). Another explanation consistent with our observations is that nematodes may additionally or alternatively, selectively feed on other microbial eukaryotes present in the sediment. The latter suggestion has been formulated before based on natural stable isotope values of intertidal and deep-sea nematodes, foraminifers and sediment POM (e.g. Iken et al. 2001; Moens et al. 2005a, Nomaki et al. 2008).

Protists

Although the experimental labeling approach has the potential to trace and quantify direct linkages between base and higher trophic levels, the transfer pathways and organisms involved remain unknown (Middelburg et al. 2000). This also applies to fatty acid and natural stable isotope analyses when performed on certain organisms without simultaneously analyzing the fatty acid composition and isotope signatures of their potential food sources. In our study this resulted in speculations on potential intermediate food sources between phytodetritus originating from the euphotic layer and deep-sea nematodes, despite the strong bentho-pelagic link detected by both fatty acids and dual stable isotopes along the ANDEEP-SYSTCO transect in the Southern Ocean (**Chapter 4**). Both the potential of bacterivory as an answer to the minute, or lack of short-term response of nematodes in tracer experiments, and its importance in deep-sea nematode diets on longer terms was, however, shown to be minimal (**Chapter 2 and 4**).

In estuarine and coastal marine systems, both benthic and pelagic protozoa, especially flagellates and ciliates, are important grazers of bacterial and phytoplankton production and capable of consuming DOM (Ederington et al. 1995 and references therein; Sherr 1988; Fenchel 1968). A grazing experiment performed on isolated and cultured benthic ciliates and nematodes has shown that ciliates in turn are grazed upon by a predatory nematode (Hamels et al. 2001). Nematode scavenging on dead foraminifers has been observed by Moens and Vincx (1997). However, overall there is remarkably little known about links between other benthic protozoa and higher trophic levels. Natural observations and counts, environmental RNA surveys and pyrosequencing have shown that both groups of microbial eukaryotes are present and phylogenetically diverse at both chemosynthetic and photosynthetic driven deep-sea ecosystems (e.g. Arndt et al. 2003; Takishita et al. 2010; Pawlowski et al. 2011). In Mediterranean deep-sea sediments, flagellate density was also significantly correlated to bacterial density (Danovaro et al. 1998). Therefore, in deep-sea sediments, ciliates and flagellates may comparably provide a mechanism by which bacterial production, phytodetritus or DOM is transferred to higher trophic levels. A similar function may be fulfilled by foraminiferans. The well-established taxonomic groups include mainly multi-chambered calcareous Rotaliida and agglutinated Textulariida, whose tests are well preserved in sediment samples. However, the majority of the foraminiferal identities found in the pyrosequencing study of Pawlowski et al. (2011) on Arctic and Antarctic deep-sea sediments, belonged to the non-identified groups of monothalamous (single-chambered) taxa or clades of which the morphology and biology is unknown. They are probably tiny, having no theca or organic one, such as the allogromiids (Gooday 2002b). Preservation and recognition of these organisms requires careful sampling, handling and sieving of the sediment. The centrifugation technique applied for the extraction of metazoan meiofauna is inappropriate to quantitatively extract

any of the above mentioned protists. Due to these methodological problems the presence as well as the potential contribution to carbon processing of these fragile protists in deep-sea sediments has not been quantified yet.

PARADIGM OF DEEP-SEA NEMATODE DIVERSITY

The highly heterogeneous nature of deep-sea habitats lies at the base of the high diversity of organisms found there. Mechanisms that underlie species coexistence, however, seem to differ between different deep-sea ecosystems. Defined according to basic energy resources, for example, chemosynthetic and photosynthetic driven deep-sea ecosystems harbor very different benthic communities in terms of diversity, composition and standing stock. While chemosynthetic driven ecosystems are considered as food-rich environments, metazoan benthic diversity is generally low. On the contrary, while photosynthetic driven deep-sea ecosystems are considered as food-poor, diversity is relatively high. Food availability is, on the other hand, generally positively related to benthic standing stocks. It is clear that for both types of ecosystems, other environmental forces act as main drivers of local and regional diversity.

Chemosynthetic driven ecosystems

There are numerous types of geologic features created by seepage or venting on the seafloor. They occur at a variety of spatial scales, from a few meters to several kilometers. The intrinsic heterogeneous physical and biological nature within and between each of these features arises from variations in the intensity, volume, chemical composition and temperature of fluid flow, the occurrence of gas hydrates, water depth, age, etc. (Cordes et al. 2010). These characteristics at least partly determine biodiversity at a local and regional scale. Organisms occurring at these reduced environments are microbia, endemics and more opportunistic colonists. Among the endemic organisms are the typical symbiont-bearing megafauna (e.g. vestimentiferan tubeworms, vesicomyid clams, and Cladorhizidae sponges; reviews by Sibuet and Olu 1998; Levin 2005). So far, no symbiont-bearing meiofauna were found at seeps or vents (Vanreusel et al. 2010a). Instead, at vents, dominant nematode genera are also present in the regular deep-sea sediments. At seeps, on the contrary, dominant species show strong taxonomic similarities with species from organically enriched sew-habitats generally don't occur in the surrounding non-seep sediments (review by Vanreusel et al 2010a). The occurrence of the dominant seep-associated *Daptonema* and some other less dominant genera (relative abundances $\geq 3\%$) as (sub)dominant genera in the surrounding non-seep sediments at both Hydrate

Ridge (**Chapter 5**), and Sagami Bay (Shirayama and Ohta 1990) indicates that oceanographic conditions, in this case an oxygen minimum zone (OMZ), add a structuring (homogenizing) effect on nematode seep assemblages. These findings favor the idea that where OMZs intersect the seafloor, oxygen stress creates a pool of species that are tolerant to low-oxygen levels; and which may colonize the seeps and further adapt to sulphidic conditions to increase their local success. Including nearby margin sediments outside the OMZ is, however, necessary to find out if the OMZ sediments favor the success of nematode species that are tolerant (colonists) or adapted (endemics) to low oxygen levels and which species additionally (colonists) or alternatively (endemics) occur at seepage sites due to lack of tolerance of most other metazoan life or a competitive successful adaptation, respectively. Overall, the nematode community at Hydrate Ridge seeps resembles deep-sea seeps worldwide based on the low nematode genera richness, low diversity and low evenness, which points to the fact that the additional chemical energy source stimulates only a few species that respond significantly to the increased food availability (review by Vanreusel et al. 2010a). While low numbers of species may be explained by the ability of only few species to adapt to the toxic environment, low evenness and high dominance may result from the opportunistic behavior of some species that competitively exclude others (Vanreusel et al. 2010a). The coexistence of nematode species in seep habitats suggests that within this severely stress constrained environment niche differentiation is still possible, probably partly due to the possibility of species-specific feeding on different resources as discussed in **Chapter 5**.

Photosynthetic driven ecosystems

Spatial patterns of biodiversity in the photosynthetic driven deep-sea environments are beginning to be clarified. Our comprehension of the mechanisms driving these patterns is, however, still poor. The more frequently invoked biological and environmental factors that influence species diversity with for example depth are: sediment grain size and substrate heterogeneity; productivity, organic content or microbial features; food resources; oxygen availability; current regimes; and catastrophic disturbances (Danovaro et al. 2010 and references therein). For each biotic benthic group or for each phylum or lower taxonomic level, these factors can act in different combinations and can overwhelm other local or regional factors, thus causing complex and unpredictable responses (Levin et al. 2001). Small-scale experiments preferably performed in situ, therefore, form the first step to elucidate taxon-specific responses to controlled environmental factors and as such the underlying processes that drive diversity.

The amount of available energy seemed to have a positive impact on meiobenthic taxon richness in, for example, the deep Mediterranean (Gambi and Danovaro 2006) and on nematode species richness over latitudinal scales in the deep North Atlantic and the central equatorial Pacific (Lambshead et al. 2000, 2002). The relatively high diversity of nematode species in deep-sea sediments is also often attributed to a high level of niche specialization with food as an important driving factor. Therefore, it is hypothesised that high diversity in morphological features (e.g. size and shape of buccal cavities, lips, appendages, etc.), different digestive efficiency or feeding movements and food preferences could be selected for by relatively low food availability demanding greater efficiency (e.g. Moens et al. 2006). On the other hand, Tilman and Pacala (1993) hypothesised that a limited spectrum of resources may lead to competitive similarity, causing competitive exclusion to occur at relatively slow rates. This could allow infrequent disturbances or stochastic recruitment to reset local species structure before exclusion occurs. According to the latter scenario, Snelgrove and Smith (2002) predict that increases in local diversity would be accompanied by increases in the numbers of ecologically similar species, and thus increased ecosystem redundancy. However, as discussed earlier, our understanding of nematode feeding behavior is generally still very poor, especially for the deep sea. Gaining knowledge on, preferably genus- or species-specific, feeding selectivity and flexibility in free-living deep-sea nematodes and their array of food sources is, therefore, essential to understand diversity patterns, functional implications of diversity, and species redundancy within trophic levels.

In conclusion, the coexistence of multiple species of closely related taxa in environments that appear homogeneous (at different scales, within different ecosystems) suggests that the organisms experience and partition habitats and niches based on sources of heterogeneity that we cannot see or detect with our current tools, or that non-equilibrium dynamics are more important than we appreciate (Levin et al. 2010b). The studies presented in this thesis, both on chemosynthetic (**Chapter 5**) and photosynthetic driven ecosystems (**Chapter 2, 3 and 4**) acknowledge and discuss the possibility of resource partitioning among deep-sea nematode species and recognize the limitations of our current tools and techniques to elucidate species-specific feeding behavior and potential resource-partitioning. The role of non-equilibrium dynamics in maintaining diversity, on the other hand, was demonstrated by the in situ performed colonisation experiment at the HAUSGARTEN site (**Chapter 3**). In accordance to the onboard performed experiment of Gallucci et al. (2008b), early colonisation of disturbed patches indicated poor predictability of community composition despite similar levels of abundance and diversity. Therefore, both studies lend experimental support to the existence of a spatio-temporal mosaic which emerges from highly localized successions. Both studies also support the hypothesis that rare species or genera constitute a pool of transient potential immigrants and give evidence that they may depend on disturbance for

persistence (Pielou 1975). Similar results have been found for deep-sea macrofauna species (Desbruyères et al. 1980; Smith et al. 1986; Grassle and Morse-Porteous 1987; Snelgrove et al. 1996). In general, rare nematode genera and species (each < 2% of relative abundance) together represent around half or more of the total community in well-oxygenated, soft deep-sea sediments (e.g. Hoste 2006, Gallucci et al. 2008a). In the synthesis of Gallucci (2008), species richness was even positively related with the number of singletons (i.e. species that occur with only one individual), indicating that local patterns of deep-sea nematode species richness seem highly dependent on the pool of rare species. Together with the results from the in situ colonisation experiment (**Chapter 3**), this indicates that in order to appreciate the overall importance of any local-scale ecological process maintaining species richness and diversity of deep-sea nematode assemblages, consideration of rare species ecology is crucial.

FUTURE DIRECTIONS AND CONSIDERATIONS

The implementation of stable isotope labelling techniques in ecological deep-sea studies has considerably improved our knowledge on benthic food web interactions during the past fifteen years. Pulse-chase experiments in which ^{13}C-labeled algae were presented enabled direct observation and quantification of carbon flow pathways in the sedimentary environment. Bacteria and several macrofaunal and protist taxa contributed significantly to the short-term processing of phytodetritus. Together with flux measurements and biomass data, these isotope tracer data were applied to models that quantify biotic carbon cycle processes in the deep sea (e.g. Gontikaki et al. 2011b; van Oevelen et al. 2011). Many of the implemented observations are however sparse in time, space and considered benthic taxa. The unresolved questions on nematode food sources and metabolism rates undoubtedly affect the accuracy with which nematodes are included in these benthic food web models (Moens et al. 2004). Therefore, with the urge to understand benthic carbon dynamics and predict the response of deep-sea ecosystems to global change phenomena, there is a clear need for both carbon assimilation and respiration data of deep-sea benthic key organisms in different deep-sea environments. With key organisms I mean organisms that represent a considerable part of the benthic biomass or abundance, including bacteria, protists, meiofauna (more specific nematodes), and macrofauna.

The experimental and observational work on the feeding ecology of nematodes presented in this thesis has provided no definite answers to questions about what deep-sea nematodes directly and significantly feed on. Rather, bacteria appeared to be a component of their diet at the Hydrate Ridge seeps, and not in photosynthetically driven deep-sea

ecosystems. Instead biochemical characteristics of Southern Ocean deep-sea nematodes evidenced a clear bentho-pelagic link. For both types of ecosystems, speculations on potential food sources have been put forward that allow development of testable hypotheses.
Central to the ideas put forward is the recognition of the potential importance of DOC and small-sized protists in the diet of nematodes as well as potentially slower metabolic rates.

Besides the study of the quantity and biochemical composition of sediment OM and potential relationship with benthic taxa, a combination of isotope addition and fatty acid-specific stable isotope analysis of organisms may provide useful approaches to address these issues. Both ^{13}C-labeled DOC substances (e.g. glucose, algal amino acid mixtures, etc.) and pre-cultured algae or bacteria may be added to deep-sea sediments and quantitatively traced in as many as possible organisms, the sediment and respired CO_2. Label addition and respiration measurements should preferably be done on in situ incubation chambers, while after retrieval and fixation the organisms may be extracted and analysed for fatty acid-specific stable isotope signals. Fatty acids that are predominantly derived from bacteria are expected to have an elevated $\Delta\delta^{13}$C, as compared with the $\Delta\delta^{13}$C of compounds that are assimilated from other resources (van Oevelen et al. 2006a). When applied to the classical feeding groups according to Wieser (1953), this might reveal a great deal of the relevance of this classification system. Another option is to isolate, for example, nematodes and add labeled substances under axenic conditions while measuring respiration. This would allow to estimate if, for example, nematodes incorporate DOC in body tissues or whether they respire it immediately. Of great importance is to consider both shorter and longer time scales with sufficient sampling intervals in any of these types of incubation experiments. The main methodological constraints to overcome to make these experimental set-ups realistic are however, the sampling difficulties, limited accessibility, high costs and risks, and ship time restrictions that may lead to a reduction in both spatial and temporal resolution of the experiments. Above all, the requirement of sufficient biomass to perform the preferable biochemical analyses hampers the possibility to expand the ecological scale of the experiments (i.e. the inclusion of more benthic taxa). Mass sampling and extraction in combination with dual stable isotope and fatty acid analyses on as many as possible benthic taxa might already answer some of the speculations on potential intermediate food sources for nematodes, but implies a time consuming task.

Overall, considering the effort in the past, figuring out deep-sea nematodes' diets and metabolic rates would mean a great breakthrough and lead to a reconsideration of ocean carbon budgets.

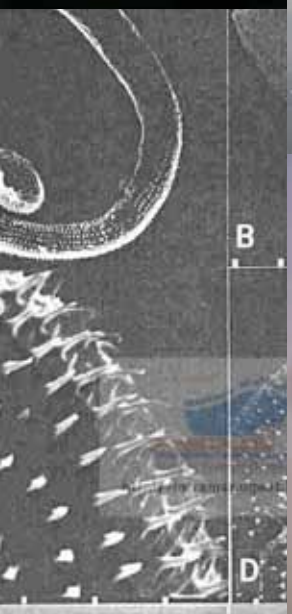
Together with the above mentioned studies on the feeding ecology of deep-sea nematodes, experimental and observational studies on patchiness at different spatial scales will improve our basic understanding of controlling processes in deep-sea biodiversity. Additionally, it will improve our presently poor ability to predict the consequences of anthropogenic impacts at the deep-sea bed. Molecular biological approaches are revealing complex and previously unseen patterns in species and genetic diversity in deep-sea environments, adding further challenges to evaluation of diversity (Snelgrove and Smith 2002). Clearly, there is much work remaining to characterize the patterns and scales of nematode species diversity on the deep-sea floor.







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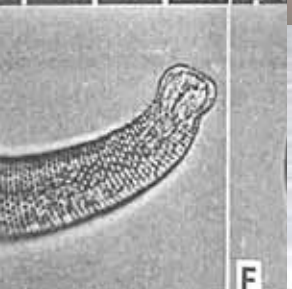
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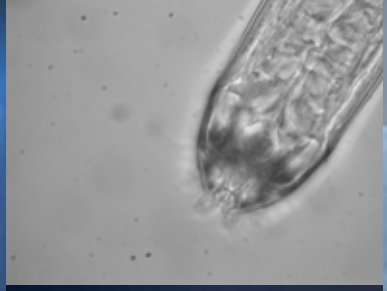










Addenda

Addendum 1

Antarctic deep-sea meiofauna and bacteria react to the deposition of particulate organic matter after a phytoplankton bloom

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ABSTRACT

During the RV Polarstern ANT XXIV-2 cruise to the Southern Ocean and the Weddell Sea in 2007/2008, sediment samples were taken during and after a phytoplankton bloom at 52° S 0° E. The station, located at 2960 m water depth, was sampled for the first time at the beginning of December 2007 and revisited at the end of January 2008. Fresh phytodetritus originating from the phytoplankton bloom first observed in the water column had reached the sea floor by the time of the second visit. Absolute abundances of bacteria and most major meiofauna taxa did not change between the two sampling dates. In the copepods, the second most abundant meiofauna taxon after the nematodes, the enhanced input of organic material did not lead to an observable increase of reproductive effort. However, significantly higher relative abundances of meiofauna could be observed at the sediment surface after the remains of the phytoplankton bloom reached the sea floor. Vertical shifts in meiofauna distribution between December and January may be related to changing pore-water oxygen concentration, total sediment fatty acid content, and pigment profiles measured during our study. Higher oxygen consumption after the phytoplankton bloom may have resulted from an enhanced respiratory activity of the living benthic component, as neither meiofauna nor bacteria reacted with an increase in individual numbers to the food input from the water column. Based on our results, we infer that low temperatures and ecological strategies are the underlying factors for the delayed response of benthic deep-sea copepods, in terms of egg and larval production, to the modified environmental situation.

Keywords: Deep-sea sediments · Meiofauna · Bacteria · Vertical migration · Benthic organic carbon flux · Sediment community oxygen consumption (SCOC) · Chloroplast pigments · Fatty acids · Southern Ocean

INTRODUCTION

Carbon fixation by phytoplankton and the subsequent cascade of grazing, export to deeper water layers, and sedimentation on the seafloor, often referred to as the "biological pump" (Longhurst and Harrison 1989), represents one of the major CO_2 sinks on earth and is the major energy source for abyssal life (e.g. Sarmiento and Le Quéré 1996; Smith et al. 2008). The intensity of primary production in the euphotic zone, often thousands of meters above the seafloor, is directly coupled to the ecosystem structure and function in the abyss (Billet et al. 1983; Gooday and Turley 1990; Graf 1989, 1992; Sibuet et al. 1989; Smith et al. 2008; Tseytlin 1987). Increasing food limitation towards the abyss leads to a shift in community biomass distribution in benthic animals from the larger macro- and megafauna, which dominate the

biomass in shallower areas, to the smaller size classes of meiofauna and bacteria (Rex et al. 2006).

Despite their low abundances in the abyssal deep sea, macrofauna may nevertheless play a considerable role in the initial processing of fresh phytoplankton from the water column (Levin et al. 1999; Moodley et al. 2002; Witte et al. 2003a, b). Nonetheless, bacteria are the most important component for remineralization, and react immediately (Cahet and Sibuet 1986; Moodley et al. 2002; Witte et al. 2003a) or with a distinct time lag (depending e.g. on water depth; Witte et al. 2003b) to the experimental deposition of organic matter. Likewise, foraminifera are important for both remineralization and as an intermediate link in the energy flow from phytodetritus to small metazoans (Gooday et al. 1996; Koho et al. 2008; Moodley et al. 2002; Nomaki et al. 2008; Witte et al. 2003b; Würzberg et al. 2011a).

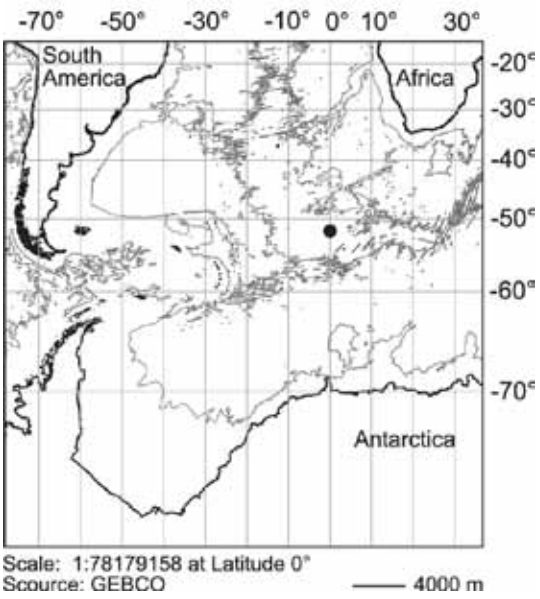
The smallest components of metazoan life in the deep sea are meiofauna, benthic animals classically defined as passing a 1 mm mesh and being retained on a 32 µm mesh. In shallow-water habitats, meiofauna can contribute significantly to the regulation of benthic turnover, and also serve as food for secondary consumers (Coull 1988; Giere 1993). The reaction of deep-sea metazoan meiofauna to food input has been investigated in several observational and experimental studies (Baguley et al. 2008; Danovaro et al. 2000; Gooday et al. 1996; Guilini et al. 2010; Ingels et al. 2010b; Moodley et al. 2002; Shimanaga et al. 2000; Witte et al. 2003b). These studies have reported a lack of response or time lags of several days to months between food presentation and measurable reactions, such as incorporation of food or reproduction. Although respiration rates and oxygen consumption of benthic communities (SCOC) almost immediately increase when fresh food is available (e.g. Witte et al. 2003b), there is no evidence that meiofauna is involved in this. To date, only a few studies that included the reaction of metazoan meiofauna to food input have been carried out for abyssal depths (e.g. Baguley et al. 2008; Cahet and Sibuet 1986; Guilini et al. 2011; Ingels et al. 2010b; Witte et al. 2003b).

While still very little is known about the benthic microbial communities in the Southern Ocean (De Wit et al. 1997), meiofauna communities of Antarctic deep-sea regions have been studied more intensely in recent years (Gutzmann et al. 2004; Herman and Dahms 1992; Vanhove et al. 1995, 2004). However, both the reasons for their success and their role as a potential trophic link between sedimented organic matter and higher-level consumers are still not known.

During the RV Polarstern ANT XXIV/2 expedition to the Southern Ocean, the aim of the ANDEEP-SYSTCO project (ANtarctic benthic DEEP-sea biodiversity: colonisation history and recent community patterns - SYSTem COupling; Brandt et al. 2011b) was to link biological processes occurring in the water column to the functioning of benthic compartments of the

deep Southern Ocean. Therefore, a deep-sea station located at the 0° meridian at 52° S (Fig. 1) was sampled while a phytoplankton bloom was in progress in the water column, and the station was revisited after the remains of the bloom had settled to the ocean floor (detectable as a layer of greenish fluff on the sediment surface). We were able to obtain measurements of the benthic C-flux, oxygen penetration depth, oxygen consumption, pigment content, and fatty acid composition in the sediment; bottom-water particulate organic matter (POM); and bacterial and metazoan meiofauna abundances. Thus, it was possible to obtain an integrated view of the response to a phytodetritus fall on the Southern Ocean deep-sea floor.

Figure 1. Map showing the location of stations 13 and 85 at 52° S 0° E in the Southern Ocean.



In view of the findings of previous studies on the population response of deep-sea meiofauna to food input (e.g. Danovaro et al. 2000), we did not expect metazoan meiofauna to react with an immediate increase of reproduction to the enhanced organic matter supply directly after the settling event. However, because the reaction of meiofauna to food input had not been investigated previously in the Southern Ocean, we looked for changes in reproductive effort that might be triggered by the sudden input of food in this otherwise adverse environment. Copepods, with their externally carried egg sacs and their easily recognizable offspring, the nauplius larvae, perfectly meet the requirements for a model organism to study the reaction of benthic meiofauna communities.

With our investigation we wanted to test the following hypotheses:

Meiofauna and bacteria in oligotrophic Antarctic deep-sea environments react immediately to seasonally deposited food input with (1) enhanced locomotor and respiratory activity, and (2) observable reproductive effort (increases in number of bacterial cells, production of eggs in copepods, and number of copepod nauplius larvae).

MATERIAL AND METHODS

Selection of study area and study dates

During the RV Polarstern ANT XXIV/2 expedition, continuous measurements of chlorophyll-a content in the upper water column were carried out using standard methods based on fluorescence. When enhanced chlorophyll a values indicated a local phytoplankton bloom (Herrmann and Bathmann 2010), we decided to start the sampling at this position in order to have a sufficient time lag between the initial sampling and revisiting the station on the return leg to South Africa. The selected station was located at 52° S, between the Polar Front (PF) and the South Antarctic Circumpolar Current Front (SACCF) (Fig. 1).

The first deep-sea sediment sampling took place on 5 and 6 December 2007, during the phytoplankton bloom (Station PS 71/13, from now on referred to as "station 13", between 52°0.41' S, 0°1.08' E and 52°2.25' S, 0°1.11' W, 2963 - 2990 m). The site was revisited and sampled again on 26 and 27 January 2008, 52 days after the bloom was observed in the surface water layer (Station PS 71/85, from now on referred to as "station 85", between 52°1.14' S, 0°0.07' E and 52°1.53' S, 0°0.16' E, 2964 - 2995 m; see also Table 1). The gear deployed for this study included a multicorer, a free-falling lander system equipped with oxygen microprofiler systems, and a carousel bottle water sampler.

Table 1. Deployments of the lander and the multicorer (MUC) at the 52° S station during the SYSTCO expedition (RV Polarstern cruise ANT XXIV-2). Sampling date, position (latitude and longitude), water depth (according to winch rope length), sediment description, and cores used for this study are given.

Station/Deployment/ Gear	Date	Latitude	Longitude	Depth (m)	Sediment (MUC)	Core distribution (MUC)
Station 13 (PS 71/13)						
13-2 Lander	05.12.07	52°0.41′ S	0°1.08′ E	2990		
13-12 MUC	06.12.07	52°2.22′ S	0°1.04′ W	2963	foraminiferan/ diatom ooze	3 cores meiofauna 2 cores oxygen/sediment 2 cores sediment analyses 1 syringe bacteria
13-14 MUC	06.12.07	52°2.25′ S	0°1.11′ W	2970	foraminiferan/ diatom ooze	3 cores meiofauna 2 cores oxygen/sediment 2 cores sediment analyses 1 syringe bacteria
Station 85 (PS 71/85)						
85-1 Lander	26.01.08	52°1.14′ S	0°0.07′ E	2995		
85-5 MUC	26.01.08	52°1.20′ S	0°0.20′ E	2965	foraminiferan/ diatom ooze with greenish fluff layer	3 cores meiofauna 2 cores oxygen/sediment 1 core sediment analyses 1 syringe bacteria
85-7 MUC	27.01.08	52°1.53′ S	0°0.16′ E	2964	foraminiferan/ diatom ooze with greenish fluff layer	3 cores meiofauna 1 core sediment analyses 1 syringe bacteria

In situ measurements of oxygen microprofiles

On both sampling dates, a customized free-falling lander (Geomar-type) equipped with a 3D-oxygen microprofiler at station 13-2 and a 1D-oxygen microprofiler at station 85-1 was deployed (Table 1). Electronics and pressure housings of both profilers were purchased from Unisense AS, Aarhus, Denmark, and were equipped with identical pressure-compensated Clark type microsensors, also supplied by Unisense AS. The lander stations were located in close vicinity to the stations sampled with the MUC (Table 1).

The 3D-oxygen microprofiler system is not restricted to driving sensors vertically into the sediment, but is also able to extend its sensor array (up to 12 oxygen microsensors) horizontally in order to measure cascades of microprofiles over a target area of approximately 30 x 35 cm. The 1D-microprofiler was equipped with up to 5 oxygen sensors that were pre-calibrated according to Sauter et al. (2001). During approximately 5 - 6 hours at the sea floor, the sensors were lowered through the water-sediment interface into the sediment with a vertical resolution of 0.5 mm (3D profiler) and 0.25 mm (1D profiler), respectively. Apart from the different vertical resolution (related to the available time at the sea floor), the calibration method and measuring technique of both systems were the same. Therefore, the possibility that different profile shapes originate from technical differences can be excluded. For porosity determination, a resistivity sensor (formation factor probe) was used. Additional *ex situ* oxygen measurements were made in the laboratory on shipboard, using sediment cores collected with the MUC (see Sachs et al. 2009).

Oxygen microprofiles were obtained as raw data sets (mV signal) from *in situ* measurements, which were converted to oxygen values via the following steps: Correction of sensor drift if necessary, adjustment of sensor height relative to the water/sediment interface, conversion of raw signal into oxygen concentration on the basis of a two-point calibration (bottom-water oxygen concentration by Winkler titration, and sensor zero reading from oxygen-free water).

For the determination of organic carbon fluxes to the seafloor, first the diffusive oxygen uptake was calculated from the pore-water oxygen microgradient at the sediment surface, using the software PROFILE of Berg et al. (1998). The oxygen uptake was then used to calculate the amount of carbon aerobically respired, using a modified Redfield ratio according to Anderson and Sarmiento (1994). Whereas the organic carbon flux reflects a "snapshot" of food supply at the time of measurement, the maximum depth of sediment oxygenation (oxygen penetration depth, OPD; below which the geochemical milieu becomes anoxic) changes much more slowly, on a time scale of years, in particular in the oligotrophic deep Southern Ocean. Therefore the OPD was determined as a long-term measure of carbon supply to the seafloor.

For a more detailed description of the methods see Sauter et al. (2001) and Sachs et al. (2009).

Sample collection and storage

The multicorer (MUC, internal core diameter 9.4 cm, 69.4 cm^2) was deployed twice on each sampling date (Table 1).

For the meiofauna community analysis, three cores per deployment were sliced (0 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 5, 5 - 7, 7 - 10, 10 - 15, and 15 - 20 cm sediment depth) and preserved in a borax-buffered formaldehyde-seawater solution to a final concentration of 4%. A sediment subsample for bacteria counts was taken from one core per MUC-deployment by inserting a 10-ml syringe with cut-off tip (diameter 1.4 cm) to a sediment depth of 5 cm. The syringe samples were immediately frozen at -20 °C.

One core per sampling date was immediately cut into 1-cm sediment horizons (the first centimeter was cut in two slices at 0 - 0.5 and 0.5 - 1 cm) to a depth of 25 cm and frozen at -80 °C for pigment analyses. Later, in the laboratory, these samples were stored at -30 °C. A second core was sliced (0 - 1, 2 - 5 and 6 - 10 cm) and stored at -80 °C for fatty-acid analysis. Only the solid component of this sediment core was sampled; the supernatant was discarded.

Bottom water was taken by means of a carousel bottle water sampler (Sea-Bird SBE 32). Water was sampled close to the seabed at station 13 (39 m above the seabed) and station 85 (36 m above the seabed). Samples of particulate organic matter (POM) were collected by filtering 14 (station 13) or 18 (station 85) liters of water through a precombusted (4 h for 400 °C) GF/C filter (approx. 1.2 µm retention size). Filtering was conducted at 300 mbar and stopped when a distinct coloring appeared on the filter. Two filters per station were frozen at -80 °C for analysis of fatty-acid composition.

Sediment parameters

The chloroplast pigments were analyzed in order to detect whether the sediments from stations 13 and 85 contained fresh or degraded phytodetritus. Pigment analyses were performed by high-performance liquid chromatography (HPLC). For analytical preparation, each core section was homogenized and subsamples of 1 cm^3 taken. The sediment sample was mixed with 100 µl of internal standard (canthaxanthin) and 1 ml of glass beads (1 mm diameter). This mixture was extracted 3 times with 3 ml acetone in a cell mill for 3 min. After centrifugation (10 min at 4000 rpm and 0 °C) the extracts were unified and concentrated on an Alltech C18™ solid-phase extract clean column. Pigments were eluted with 100% acetone and further concentrated under nitrogen atmosphere in the dark to a final volume of 0.3 ml. Finally, pigments were measured with a Waters™ HPLC system according to Hoffmann et al. (2006).

The composition of fatty acids contains information about the state of degradation of organic material, and many fatty acids are biomarkers for different producers and bacteria. Fatty acids were analyzed in three random subsamples (ca. 50 - 100 mg taken with a spoon) per

depth layer (0 - 1, 2 - 5 and 6 - 10 cm). Extraction was performed with minor modifications as described by Folch et al. (1957), using ultrasonic disruption in dichloromethane: methanol (v:v/ 2:1) and a washing procedure with 0.08% aqueous KCl solution. Fatty acid composition was determined, with modifications as described by Kattner and Fricke (1986). Fatty acids were converted to their methyl ester derivatives (FAME) using 3% sulfuric methanol. The composition was analyzed with a gas chromatograph (HP 6890A) equipped with a programmable temperature vaporizer injector (Gerstel® CIS4plus) and a DB-FFAP (nitroterephthalic acid modified polyethylene glycol free fatty acid phase) column. FAMEs and free alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards of known composition. For quantification of fatty acids, 23:0 was used as internal standard.

Bacteria and meiofauna sample processing

The syringe samples for bacterial counts were sliced (0 - 1, 1 - 2, 2 - 3, 3 - 4, 4 - 5 cm sediment depth) just before further analysis. Total bacterial counts were performed using acridine orange direct count (AODC) (variant from Hobbie et al. 1977). Each sediment sample of ca. 0.5 - 1 g was transferred into a sterile test tube and fixed to a final volume of 5 ml with 2% (v/v) formalin, after thawing. Pure formaldehyde was mixed with sterilized, prefiltered artificial seawater (on 0.2 µm-pore-size filters) and buffered with borax (sodium tetraborate) to a pH of 8.8 to 9. Tetrasodium pyrophosphate was then added to a final concentration of 5 mM and the sample was incubated in the dark for 15 min. The sample was then sonicated for 3 min with a 30 s interval each minute, during which it was shaken manually. After the sample was centrifuged for 3 min at a maximum of 400 rpm, the supernatant was diluted 20-fold with sterile seawater. For AODC, the sample was stained for 5 min with an acridine orange working solution of 0.025% (w/v) and filtered on a Millipore polycarbonate 0.2 µm-pore-size filter that was stained in an irgalan black solution (Van Duyl and Kop, 1990). A filter was washed 3 times with 5 ml of sterilized Milli-Q water and mounted on microscope slides. Each filter was analyzed using epifluorescence microscopy (Zeiss Axioskop 50, excitation and emission wavelengths: 450 - 490 nm and 515 - 565 nm respectively). For each slide, at least 10 optical fields were randomly chosen and observed, and at least 200 cells were counted per filter.

The fixed meiofauna samples were washed with tap water through a 32-µm mesh sieve (no coarser upper sieve was used). Meiofauna and organic material were extracted from the remaining sediment particles by centrifugation with a colloidal silica polymer as the flotation medium (H. C. Stark, Levasil 200/40%, ϱ = 1.17) and kaolin to hold back the heavier particles during decantation (McIntyre and Warwick 1984). Centrifugation was repeated three

times at 4000 rpm for 6 min respectively. After each centrifugation, the floating matter was decanted over a 32-µm mesh sieve and rinsed with tap water. The supernatant containing the meiobenthic organisms was then stained with Rose Bengal, before manual sorting to major taxon level using a Leica MZ 12.5 stereomicroscope. The numbers of individuals belonging to higher taxa, copepod nauplii, and copepods carrying eggs were counted.

For the interpretation of the vertical distribution, the numbers of bacteria and meiofauna individuals were converted into relative abundances per sediment horizon. In order to obtain these values, the sum of individuals from the complete core was taken as 100% and the percentages per slice were calculated.

The use of relative abundances for bacteria and meiofauna data

As indicated by the results for the fatty acids, there is also evidence for patchily distributed standing stocks of bacteria and meiofauna in the deep-sea area investigated. During our expedition it became clear that food availability differed on medium scales: an additional *in situ* measurement of oxygen microprofiles was carried out 12 nautical miles south of 52° S at station PS 71/84 (water depth 3004 m). On the same day, the fluxes were found to be approximately 20% lower (7.7 mg C $m^{-2}d^{-1}$) at station PS 71/84 than at station 85 (Sachs et al. 2009). There is a possibility that even at smaller scales, a slightly poorer pre-bloom food bank in the sediment at station 85 compared to the nearby station 13 had an effect on the abundances of benthic organisms. Absolute individual numbers of bacteria and meiofauna can be quite variable between different deployments, and even between the cores of the same MUC deployment (compare Tables 3, 4; for meiofauna see also Gutzmann et al. 2004). Therefore we decided to use relative abundances of bacteria and meiofauna for data presentation and statistical analyses, in order to detect differences which otherwise would be obscured by the absolute (or otherwise transformed) numbers found at stations 13 and 85.

Data analysis

Principal components analysis (PCA) was used to detect and visualize patterns in the composition of individual fatty acids in sediment and bottom-water samples (PRIMER v6 package; Clarke and Gorley 2006).

In the graphs, relative meiofauna and copepod abundances are presented as median values. The 25^{th} and 75^{th} percentile were added in order to visualize the distribution range of the data. According to this graph, a statistically significant difference could be expected between

stations 13 and 85 for the first three centimeters of the sediment. The meiofauna data for the 2 - 3 cm sediment horizon (station 13 vs station 85) did not pass the test for equal variance. A nested ANOVA (after Sokal and Rohlf 1981) was carried out to test for differences between the 0 - 1 and 1 - 2 cm sediment layers of stations 13 and 85. The use of the nested design can be explained as follows: at the two stations 13 and 85 (A) two MUC-deployments were carried out per station (B nested in A), and the data for three sediment cores per deployment were the test data. For the test for differences between the percentage of copepods with eggs per total copepods (adults and copepodids) and the percentage of nauplii per total copepods, the data for the complete cores (0 - 20 cm) were used. The nested ANOVA was carried out using the free statistical software OpenStat (http://www.statpages.org/miller/openstat/ by William G. Miller) and confirmed by a spreadsheet available from McDonald (2009; http://udel.edu/~mcdonald/statintro.html).

Multivariate meiofauna community analyses were carried out on abundance data for higher taxa and copepod nauplii from the first centimeter of sediment, using the PRIMER v6 package (Clarke and Gorley 2006). A Bray-Curtis similarity analysis was conducted and visualized with the aid of non-metric multi-dimensional scaling MDS. The factor "station" was applied for the ANOSIM analysis of similarities (one-way analysis).

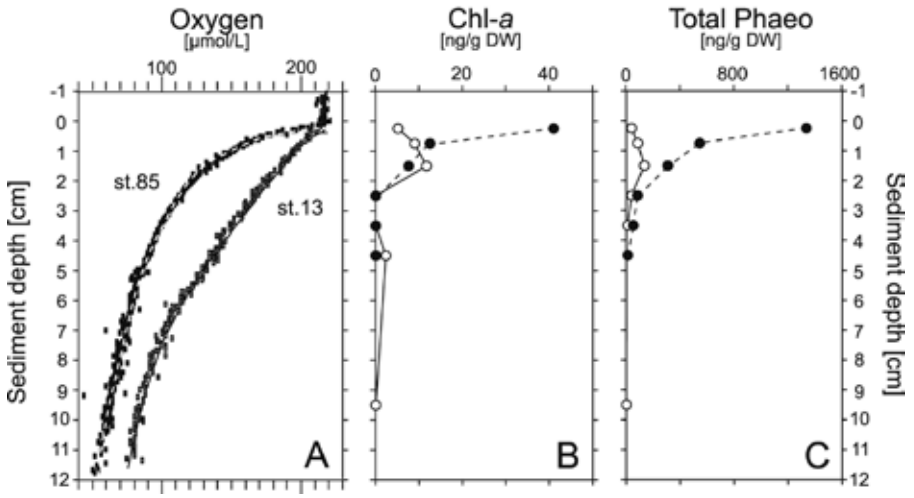
RESULTS

Sediment parameters and bottom-water POM

Phytodetritus was not detectable on the surface of the sediment cores at station 13 at the beginning of December. During the second visit, fluffy green material was observed on the sediment surface in all multicorer samples (station 85).

Chlorophyll-a (Chl-a) content at the sediment surface (0 - 0.5 cm) was eight times higher at the end of January (41.02 ng g^{-1} sediment dry mass) compared to the values measured at the beginning of December (5.13 ng g^{-1} sediment dry mass; Fig. 2B). The content of phaeopigments (Phaeo: sum of phorbid-a, p-phorbid-a, phytin-a and p-phytin-a) had increased 33 times by the end of January (1334.85 ng g^{-1} sediment dry mass) compared to the values from December (40 ng g^{-1} sediment dry mass; Fig. 2C). Pigment content gradually declined with sediment depth, as did pigment quality. Between 3 and 5 cm sediment depth, the values for the revisited station equaled those for the first sampling date.

Figure 2. Pore-water oxygen and pigment profiles at the two study locations south of the Polar Front at 52° S 0° E, ~3000 m depth: A. Oxygen content in the sediment before (station 13) and after a phytoplankton bloom (station 85), benthic C_{org} fluxes derived from (A) increased by a factor of ~3 (station 13: 3.3 mg C m^{-2} d^{-1}; station 85: 9.1 mg C m^{-2} d^{-1}); B. Content of chlorophyll-a, and C. Total phaeopigments before (white dots; station 13) and after phytoplankton bloom (black dots; station 85); DW = sediment dry mass.



Pore-water oxygenation showed a steeper decrease within the first centimeters of sediment after the organic material had settled (Fig. 2A). This indicates enhanced sediment community oxygen consumption (SCOC) after organic matter deposition (0.400 mmol O_2 m^{-2} d^{-1} at station 13 before, and 1.102 mmol O_2 m^{-2} d^{-1} at station 85 after the bloom; in situ measurements). Carbon fluxes derived from in situ oxygen measurements were moderate to low at the beginning of December (station 13; 3.3 mg C m^{-2} d^{-1}). After 7 weeks, fluxes were elevated by a factor of ~3 (station 85; 9.1 mg C m^{-2} d^{-1} after the bloom; Fig. 2A). The values obtained in situ at both stations were confirmed by laboratory measurements carried out on sediment cores from the MUC (not shown here). Whereas the fluxes differed at stations 13 and 85, the oxygen penetration depths (OPD) on both sampling dates were very similar, with 43 (±3) and 42 (±8) cm at stations 13 and 85, respectively. The OPDs of several decimeters indicated a low long-term (in terms of several years) average flux of organic matter to the seafloor at this site (see also Sachs et al. 2009).

Total sediment fatty acid content (Fig. 3) for the three analyzed sediment layers 0 - 1, 2 - 5 and 6 - 10 cm from station 13 decreased from 0.84 µg g^{-1} to 0.38 and 0.38 µg g^{-1}, respectively. For station 85, values from 1.01 µg g^{-1} to 0.25 and 0.19 µg g^{-1} were measured (µg fatty acid g^{-1}

sediment dry mass, mean of three subsamples for each layer). The ratio of long-chain (> C21) to short-chain fatty acids was low, especially in the deeper sediment layers (2 - 5 and 6 - 10 cm; Table 2). The relative content of polyunsaturated fatty acids (PUFA) was low compared to monounsaturated (MUFA) and saturated fatty acids (SFA). PUFA content was highest in the first sediment centimeter when the station was revisited (Fig. 4). The fatty acids that were generally dominant in the sediment samples were 16:0, 18:1(n-9) and 18:0, followed by 18:1(n-7) and 16:1(n-7) (Table 2).

Figure 3. Total fatty acid content of sediment layers 0 - 1 cm, 2 - 5 cm and 6 - 10 cm at stations 13 and 85 (µg fatty acid g^{-1} sediment dry mass). Mean values (box), maximum and minimum (right and left end of bar) of three subsamples per sediment horizon are given.

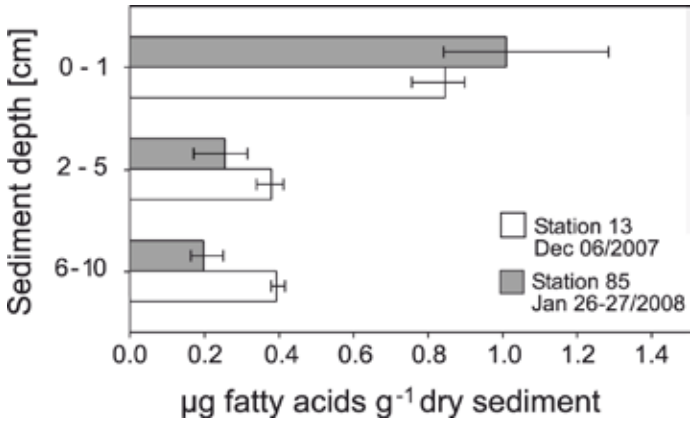


Figure 4. Proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in 0-1 cm, 2-5 cm and 6-10 cm sediment depth for stations 13 and 85. Fatty acids that contributed less than 2% of total fatty acids were not taken into account. Results are presented as % of total fatty acids. Mean values (box), maximum and minimum (upper and lower end of bar) of three subsamples per sediment horizon are given.

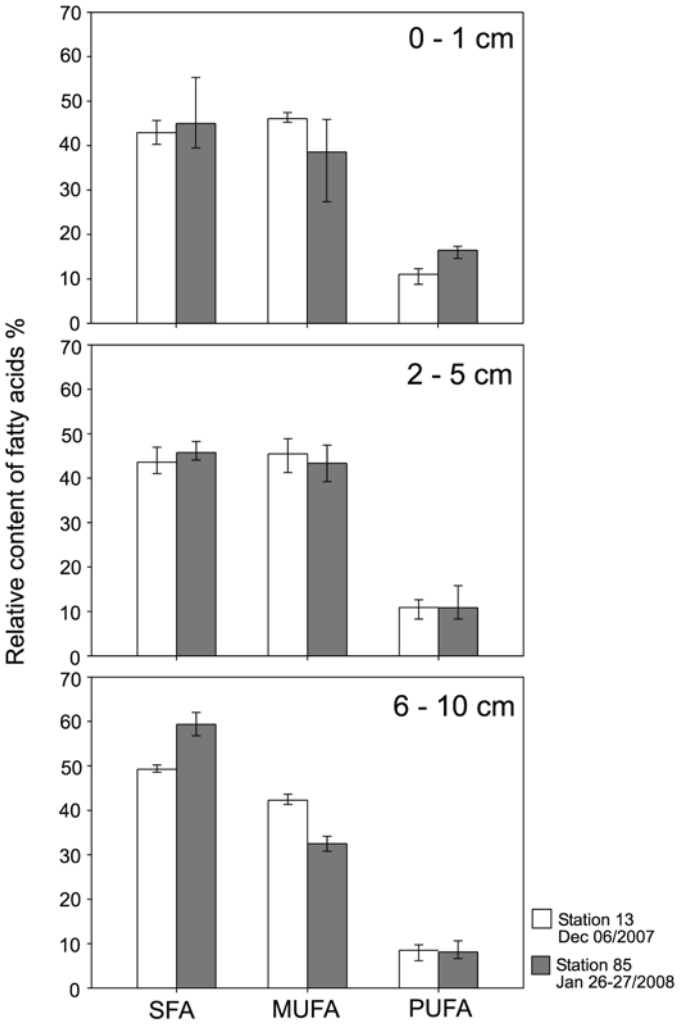


Table 2. Fatty acid composition (mean of subsamples) of bottom-water particulate organic matter (µg l^{-1}) and sediment (0 - 1, 2 - 5, 6 - 10 cm; µg g^{-1} sediment dry mass) for stations 13 and 85.

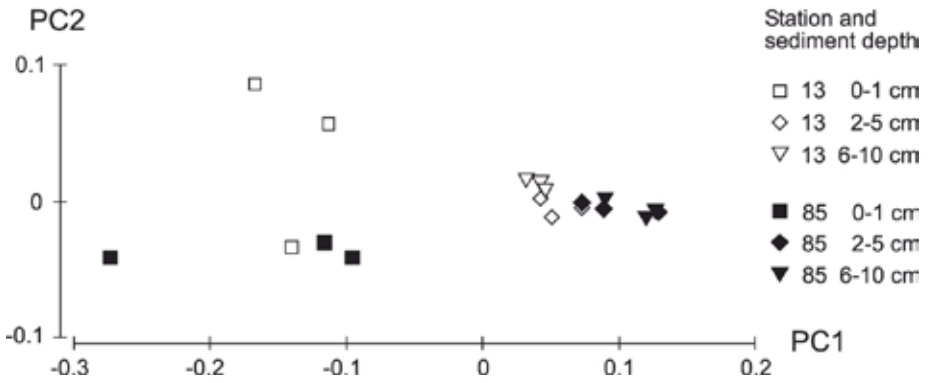
Fatty acids	Bottom water POM (µg l^{-1})		Sediment horizons (µg g^{-1} dry sediment)					
	Station 13	Station 85	Station 13: 0 - 1 cm	Station 13: 2 - 5 cm	Station 13: 6 - 10 cm	Station 85: 0 - 1 cm	Station 85: 2 - 5 cm	Station 85: 6 - 10 cm
14:0	0.004	0.0026	0.0206	0.0183	0.0176	0.0392	0.0101	0.0089
15:0	0.003	0.0016	0.0191	0.0085	0.01	0.0253	0.0061	0.0053
16:0	0.0256	0.0218	0.2115	0.0989	0.1097	0.234	0.0725	0.0633
16:1(n-7)	0.0004	0	0.0565	0.042	0.0168	0.0534	0.0216	0.0082
16:1(n-9)	0.0067	0.0019	0.0419	0.0263	0.0262	0.035	0.0198	0.0134
17:0	0.0013	0.0006	0.0203	0.0057	0.0114	0.0426	0.0032	0.0033
18:0	0.0161	0.0162	0.1389	0.0438	0.0447	0.1571	0.029	0.0269
18:1(n-7)	0	0	0.0673	0.0283	0.0218	0.0827	0.0167	0.0067
18:1(n-9)	0.01	0.0062	0.1889	0.0622	0.0873	0.1477	0.0432	0.0363
18:2(n-6)	0.0024	0.0023	0.0299	0.0221	0.0241	0.0613	0.0117	0.0109
20:4(n-6)	0	0	0.0171	0.0053	0.0106	0.0378	0.0083	0.0046
20:5(n-3)	0.0007	0.0026	0.0169	0.0057	0.0038	0.052	0.0079	0.0066
22:5(n-3)	0.0017	0.0061	0.0164	0.0103	0.0087	0.034	0.0041	0.0026
22:6(n-3)	0.0008	0.0023	0	0.0014	0	0.0075	0	0

Phytoplankton fatty acid markers (e.g. 20:5(n-3), 22:6(n-3)) were detected in higher amounts in the first centimeter after the phytoplankton bloom occurred (Table 2). The ratio of 18:1(n-9) to 18:1(n-7) was higher on the first and lower on the second sampling date after the phytoplankton bloom. The bacterial marker fatty acids 15:0 and 17:0 were most abundant in the first sediment centimeter at station 85.

The findings for the composition of the individual fatty acids were visualized by a PCA (Fig. 5), where the two first components (PC1, PC2) together explained 93% of the variation. The main contributors that explained the separation of the sediment samples from stations 13 and 85 were 16:0 (-0.615), 18:1(n-9) (-0.489), 18:0 (-0.484) and 18:1(n-7) (-0.223) for PC1, and 18:1(n-9) (-0.788), 18:1(n-7) (-0.302), 18:2(n-6) (-0.273), and 20:5(n-3) (-0.253) for PC2. The clearest differences were detected between the samples of the first centimeter of sediment of the two stations.

The total fatty acid content of the bottom-water POM samples was similar on both sampling dates (station 13: 0.073 µl/l^{-1}, station 85: 0.064 µl/l^{-1}). The most abundant fatty acids were 16:0, 18:0, 18:1(n-9), but during the second visit (station 85), the phytoplankton marker fatty acids (e.g. 20:5(n-3), 22:6(n-3) were more abundant (Table 2).

Figure 5. Principal components analysis of the individual fatty acid composition of the sediment (0 - 1 cm, 2 - 5 cm, 6 - 10 cm) at stations 13 and 85. The first two components together explain 93% of the variation observed (PC1 = 86.8%, PC2 = 6.1%).



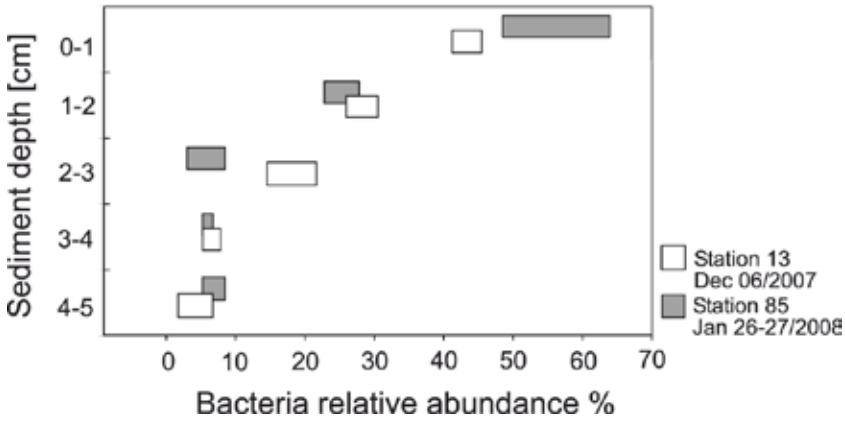
Bacterial counts

In total, the first station 13 had a higher standing stock of bacteria (7.92×10^8 and 1.47×10^9 cells in 5 cm^{-3} sediment from 0 - 5 cm depth) than station 85 (6.47×10^8 and 3.35×10^8 cells in 5 cm^{-3} sediment from 0 - 5 cm depth). Station 85 showed, in all except three sediment horizons (sample 85-5, 0 - 1 cm: 4.12×10^8 cells cm^{-3}; 4 - 5 cm: 3.37×10^7 and 2.85×10^7 cells cm^{-3}), lower numbers of bacteria than the samples from station 13 (Table 3). Bacterial numbers gradually decreased with sediment depth in the two cores from station 13, but the decline was not as abrupt as in the cores from station 85. The plot of the relative abundances of bacteria versus sediment depth (Fig. 6) shows that after the blooming event at the end of January, a higher percentage of bacteria was found in the upper centimeter of the sediment compared to the first sampling date. At station 85 the relative bacterial density reached a minimum in the 2 - 3 cm layer, whereas the minimum at station 13 was reached in deeper horizons.

Table 3. Total number of bacteria per cm³ sediment from 5 sediment horizons sampled at 52° S 0° E (2963 - 2970 m) during the Polarstern cruise ANT XXIV-2: station 13 (2 deployments), 12/06/2007, before the phytoplankton bloom reached the sea floor, and station 85 (2 deployments), 01/26-27/2008, after the bloom had settled.

Total no. of bacteria per cm³ sediment	**Station Deployment**			
Sediment depth (cm)	**13-12**	**13-14**	**85-5**	**85-7**
0 - 1	3.57E+08	6.08E+08	4.12E+08	1.62E+08
1 - 2	2.06E+08	4.46E+08	1.47E+08	9.30E+07
2 - 3	1.15E+08	3.17E+08	1.97E+07	2.82E+07
3 - 4	6.00E+07	7.70E+07	3.45E+07	2.26E+07
4 - 5	5.29E+07	2.72E+07	3.37E+07	2.85E+07
Total in 5 cm³	7.92E+08	1.47E+09	6.47E+08	3.35E+08

Figure 6. Relative abundances of bacteria in 5 sediment horizons at 52° S 0° E (one subsample taken from a MUC-core per deployment): station 13 (white boxes), before the decaying phytoplankton reached the sea floor; station 85 (gray boxes), after the settlement event. Left and right border of boxes represent the two values obtained per horizon and station.



Meiofauna abundances and community analysis

The total number of meiofauna organisms varied between 10,120 and 4,111 individuals per core (0 - 20 cm sediment depth). At station 13, a median of 788.14 ind. 10 cm^{-2} (min. 662.99 ind. 10 cm^{-2}; max. 1458.26 ind. 10 cm^{-2}) and at station 85 a median of 658.95 ind. 10 cm^{-2} (min. 592.38 ind. 10 cm^{-2}; max. 725.09 ind. 10 cm^{-2}) was counted for the complete sediment profiles (Table 4). There was no statistical difference between the total number of individuals on the two sampling dates (nested ANOVA B(A)).

Table 4. Total individual numbers of meiofauna, major meiofauna taxa, copepods with egg sacs and copepod nauplii per multicorer (MUC) core (69.4 cm^2) from six cores per station (0 - 20 cm sediment depth, minima (Min) and maxima (Max) included). Two MUC deployments were carried out on each sampling date at 52° S 0° E (2963 - 2970 m): station 13 (12/06/07) before and station 85 (01/26-27/08) after the decaying phytoplankton reached the sea floor.

Individuals per core	13-12-2	13-12-6
Acari	2	0
Amphipoda	0	0
Annelida	22	38
Bivalvia	1	0
Copepoda (adults and copepodids)	228	336
(of the adults) copepods with egg sac	5	7
Copepod nauplii	308	468
Cumacea	0	0
Gastrotricha	7	3
Gastropoda	0	0
Isopoda	1	1
Kinorhyncha	3	2
Loricifera	1	3
Nematoda	3909	4403
Ostracoda	36	37
Rotifera	64	19
Tanaidacea	1	3
Tantulocarida	0	1
Tardigrada	10	2
Indet.	8	6
Total individuals	4601	5322
% Copepods with eggs	2.19	2.08
% Nauplii per total of adults and copepodids	35.09	39.29

Station - deployment - core number									
13-12-12	**13-14-1**	**13-14-4**	**13-14-9**	**85-5-2**	**85-5-3**	**85-5-11**	**85-7-1**	**85-7-4**	**85-7-8**
0	1	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	1	0
36	54	43	36	65	70	49	38	51	36
1	2	2	1	2	0	2	2	0	0
250	339	335	192	313	268	249	274	245	201
11	9	6	7	11	5	3	2	2	4
410	534	540	339	643	488	348	388	351	406
0	0	0	0	0	1	0	0	0	0
8	7	9	4	16	12	11	11	5	5
0	0	0	0	0	0	0	0	0	1
3	4	3	3	5	1	2	3	3	1
0	4	1	0	5	6	5	1	1	0
2	5	4	2	4	8	6	4	1	8
4840	8528	5698	4015	3886	3210	4024	3882	3706	3408
31	36	27	28	40	26	24	44	21	18
30	58	78	34	21	10	20	2	18	8
0	1	3	2	1	0	3	3	5	3
2	4	2	0	8	119	3	24	47	2
4	4	1	1	17	17	13	7	3	3
0	539	8	11	6	37	12	3	2	11
5617	10120	6754	4668	5032	4273	4772	4686	4460	4111
4.4	2.65	1.79	3.65	3.51	1.87	1.2	0.73	0.82	1.99
64	57.52	61.19	76.56	105.43	82.09	39.76	41.61	43.27	101.99

Nematoda was the most abundant taxon, with a mean relative abundance of 83.1%, followed by copepod nauplii (counted separately from adults and copepodids in order to elucidate ecological aspects, 8.1%) and Copepoda (including adults and copepodids, 5%). Other taxa found were Annelida, Ostracoda, Rotifera, Tantulocarida, Gastrotricha, Tardigrada, Loricifera, Isopoda, Kinorhyncha, Tanaidacea, Bivalvia, Acari, Amphipoda, Cumacea and Gastropoda (listed in order of decreasing total numbers of individuals, with a maximum of 0.84% of occurrence).

After the phytodetritus fall (station 85), the relative abundance of metazoan meiofauna in the first sediment centimeter was significantly higher than before (Fig. 7). This finding is supported by the results of the nested ANOVA (results for A (stations): $p = 0.001$; Table 5). Additionally, meiofauna individual numbers decreased more steeply within the first three centimeters in most of the cores from station 85, compared to the earlier sampling date at station 13 (Fig. 7). Nested ANOVAs for the 0 - 1 cm sediment horizon showed that Nematoda were mainly responsible for the observed pattern (among stations $p = 0.005$), while Copepoda (Fig. 8; adults and copepodids, among stations $p = 0.83$) and copepod nauplii (among stations $p = 0.163$) did not show a significant change in relative abundances at the sediment surface.

Table 5. Results of the ANOVA (nested design with (A) groups and B(A) subgroups within group) of the relative abundances of meiofauna at the sediment surface (0 - 1 cm). A: stations 13 and 85 (groups); B: two MUC deployments at each station (nested within A); measurements: data from three MUC cores per deployment.

	Sum of Squares	Degrees of freedom	Mean *D* Square	F	p
A (groups)	415.6233	1	415.6233	857.9940	**0.001163**
B(A) subgroups within groups	0.9688	2	0.4844	0.005226	0.9948
within subgroups	741.5278	8	92.6910		
Total	1158.1199	11			

Figure 7. Relative meiofauna abundances in 9 sediment horizons from MUC cores sampled at 52° S 0° E: station 13 (white boxes, 6 MUC-cores included) before the phytoplankton bloom reached the sea floor, and station 85 (gray boxes, 6 MUC-cores) after the settlement event. Boxes indicate median value (vertical black line within the box) and 25th and 75th percentile (left and right border of box).

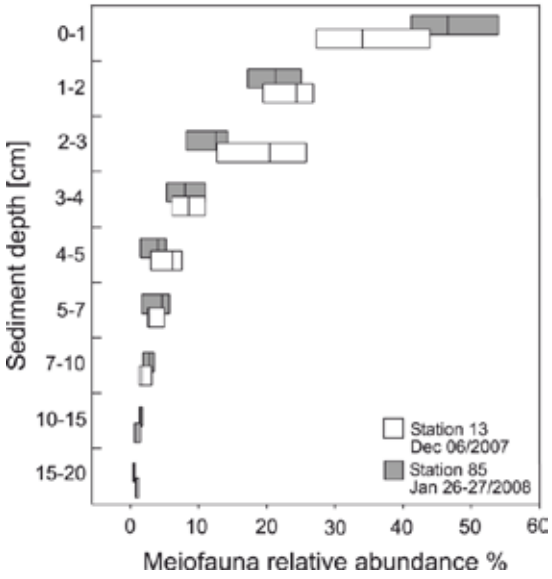
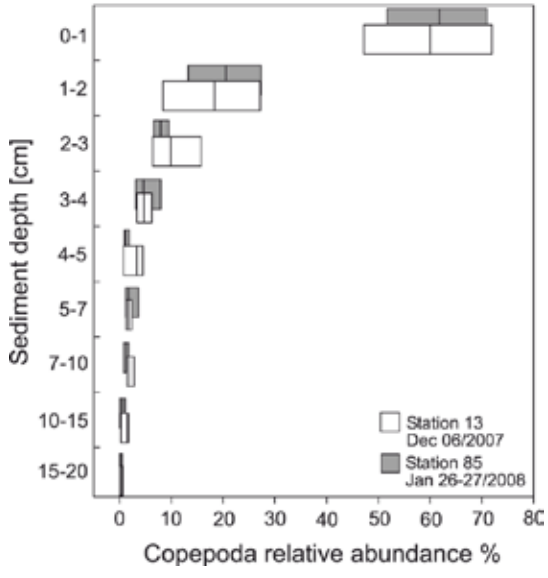
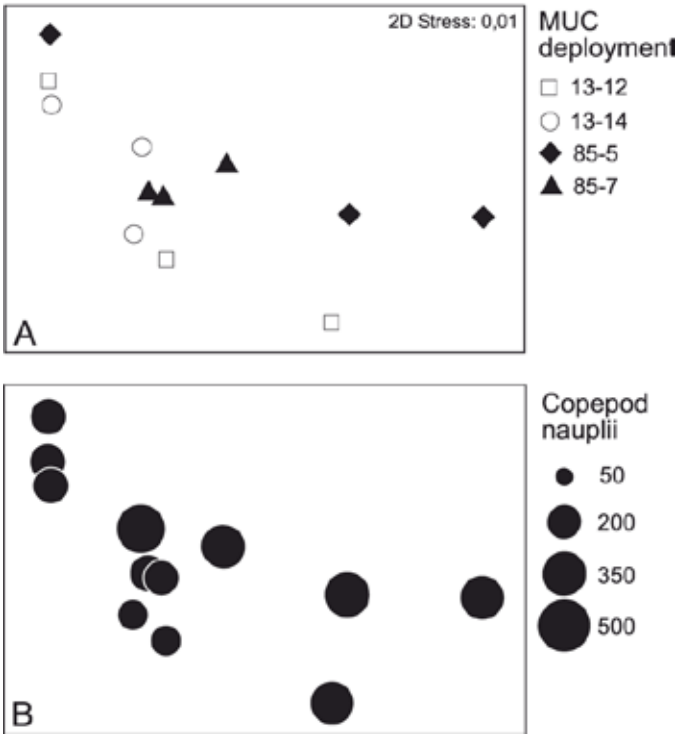


Figure 8. Relative copepod abundances in 9 sediment horizons from multicorer (MUC) cores sampled at 52° S 0° E: station 13 (white boxes, 6 MUC-cores included) before the phytoplankton bloom reached the sea floor, and station 85 (gray boxes, 6 MUC-cores) after the settlement event. Boxes indicate median value (vertical black line within the box) and 25th and 75th percentile (left and right border of box).



The meiofauna community composition (0 - 1 cm horizon, standardized by total) also did not change after the phytoplankton bloom settled (Fig. 9). This result was confirmed by an analysis of similarity (ANOSIM) (absolute individual numbers; Global R = 0.067; p = 0.22). Although the highest nematode densities were observed at the beginning of December 2007 (Station 13, Table 4), there was no influence on the meiofaunal community structure as a whole.

Figure 9. A. Multi-dimensional scaling of the Bray-Curtis similarity of the meiofauna community data (standardized by total) from the 0-1 cm sediment horizon of single multicorer (MUC) cores sampled at 52° S 0° E before (station 13) and after (station 85) the decaying phytoplankton reached the sea floor. B. Individual numbers of copepod nauplii per core, shown as bubble plot.



For the copepods, the second most abundant taxon after the nematodes, favorable changes in the environment did not lead to a visual increase of the reproductive effort during the experimental period. Copepods with eggs (Fig. 10), expressed as a percentage of total copepods (Table 4), were fewer in January (median 1.54%) than in December (median 2.42%). The nauplii, the first larval stages of copepods, did not increase in total number either, but their percentage as compared to total copepods was slightly higher after the blooming event (Table 4). However, the nested ANOVA (B(A)) for the percentage of copepods with eggs per total copepods and the percentage of nauplii per total copepods did not detect statistically significant differences between station 13 and station 85.

Figure 10. Female copepod of the genus Mesocletodes (Argestidae) with egg sac containing four large eggs attached to the abdomen. The individual was collected with the MUC at station 85 (1 - 2 cm sediment depth) and belongs to Mesocletodes sp. 32, a widely distributed species of deep-sea copepod (Menzel et al. 2011). The specimen was stained with Congo red and observed as described by Michels and Büntzow (2010); photograph by Jan Michels.

DISCUSSION

Changes in the sediment

The sediments of the abyssal Southern Ocean are generally well oxygenated and show large OPDs down to several decimeters in abyssal sediments of the Weddell Sea and the southeastern South Atlantic (Sachs et al. 2009). Our measurements at station 13 and 85 are no exception to these observations, with 43 and 42 cm OPD, respectively. Oxygen penetration depth (OPD) is a long-term indicator for organic carbon supply to the ocean floor. Thus, the variation in pore-water oxygen depth distribution obtained by the measurement of oxygen microprofiles was important for the understanding of input and recycling of organic material into this deep-sea system.

Different carbon fluxes derived from oxygen measurements before and after the phytoplankton bloom coincided with comparable OPDs on both dates. The observed bloom at the beginning of December and the concurrent carbon flux measurements at the seafloor clearly showed the correlation of surface production with benthic fluxes. Topographic characteristics of the sediment surface at station 13 may have accounted for an observed subsurface variability of the oxygen profiles from the first station (3D profiler). This is, however, unlikely, as the meiofaunal and bacterial distributions of relative abundances with depth followed the same patterns as the oxygen profiles at both stations (see below).

Phytodetritus forms aggregates that can settle at a rate of 100 - 150 m per day (Gooday 1993 and references therein). The results of the pigment analyses clearly showed that the phytoplankton bloom observed in the water column at the beginning of December had reached the seafloor at 3000 m water depth by the end of January. The high concentration of chlorophyll-a and the amount of material indicated that there was a relatively short time span between the settlement and our sampling. We assume that the phytodetritus reached the seafloor 3 to 28 days before station 85 was sampled. Three days is the minimum period reported for the onset of sediment community respiration (Witte et al. 2003b), and 28 days is the maximum time span that can be assumed if settlement started immediately after the first visit (52 days before), with a mean sinking rate of 125 m day^{-1} (according to Gooday 1993) to a water depth of ~3000 m. As expected, pigment content declined with sediment depth.

The fatty acid content of the deeper sediment layers (2 - 5 and 6 - 10 cm) was higher at station 13 than at station 85. Deeper sediment layers reflect the input of organic matter during previous sedimentation events. This finding indicates a patchy settlement of organic matter in the region, with higher input of organic material at station 13 in previous years. Patchily distributed standing stocks of bacteria and meiofauna in the deep-sea area investigated may

explain these findings (compare 2.6). However, the difference between the fatty-acid content observed in the first centimeter and the amount in the deeper sediment layers was more pronounced at station 85 than at station 13. On the one hand, the high content of fatty acids in the first centimeter of station 85 could be explained by the recent input of organic material. On the other hand, the discrepancies between station 85 and station 13 regarding the deeper sediment layers indicate a lower organic input during previous sedimentation events at station 85. The total fatty-acid content of the bottom-water POM samples showed no strong differences between both sampling dates, and was in the same range as values measured at a depth of 3000 meters in the Bellingshausen Sea (Antarctica) by Fileman et al. (1998).

Fatty acids are selectively degraded in the marine environment (Reemtsma et al. 1990), and the total concentration of fatty acids in the sediment is markedly reduced with water depth. However, in our study, considerable levels of labile fatty acids were found at the deep-sea floor, originating from the sedimentation of partially degraded material from surface water layers into the deep ocean (Fileman et al. 1998). The ratio of long-chain (> C21) to short-chain fatty acids is, because of degradation processes, generally low in marine sediments (Naganuma et al. 2001). As expected, degradation was most pronounced in the deeper sediment layers (2 - 5 and 6 - 10 cm). The higher content of polyunsaturated fatty acids (PUFA) during the second visit indicates the increased abundance of components rich in these fatty acids derived from animal or plant remains. Generally, PUFA are synthesized almost exclusively by plants (Brett and Müller-Navarra 1997).

Although the organic fluff layer on top of the sediment was not included in the fatty-acid analysis, enhanced signals of phytoplankton fatty-acid markers (20:5(n-3), 22:6(n-3)) were detected in the first centimeter of the sediment at station 85. Together with the clearly increased amounts of these fatty acids in bottom-water POM during the second visit, this substantiates the visual findings and the results from the pigment analyses. Accordingly, the degree of degradation of fatty acids (represented by the ratio of 18:1 (n-9) to 18:1 (n-7)) was higher before the input of the fresh organic matter.

Heterotrophic bacteria are particularly abundant in marine sediments (Sargent et al. 1987) and colonize and decompose settling particulate matter (Azam et al. 1983; Pfannkuche and Lochte 1993). These bacteria synthesize odd-numbered branched fatty acids such as 15:0, 17:0, 15:1 and 17:1, and large amounts of 16:1(n-7) (Dalsgaard et al. 2003 and references therein). Additionally, aggregates of phytodetritus harbor a rich bacterial and cyanobacterial flora (Gooday 1993 and references therein). Therefore, increased numbers of bacteria were to be expected for station 85 when the increased amount of bacterial-marker fatty acids 15:0 and 17:0 is considered. However, the patchy distribution of bacterial cells (compare 2.6) did not completely reflect the findings from the fatty acids.

Reaction of bacteria to food input

The absolute numbers of bacteria found in the sediment samples during this study were comparable to the lower end of previous reports from the deep sea (e.g. Rex et al. 2006; Danovaro et al. 2008a). The higher relative abundances in the uppermost sediment horizon after the bloom settled could be the result of higher bacterial growth fostered by the change in nutrient conditions on the sediment surface. However, a possible additional input of bacterial cells associated with descending phytodetritus (e.g. Grossart et al. 2006; Pfannkuche and Lochte 1993) must be taken into account. Thus, our hypothesis that bacteria in oligotrophic Antarctic deep-sea environments react with an immediately detectable reproductive effort to unexpected food input cannot be validated conclusively.

Recent findings indicate that reproduction may not be the first reaction of Antarctic benthic deep-sea bacteria to food input (D. Pearce, pers. comm.). Bacteria at subantarctic deep-sea sites near Crozet Island did not increase in number, but rather enhanced their activity in areas with high organic-carbon input. In our study, the pore-water oxygenation showed a steep decrease within the first centimeters of sediment after the phytoplankton bloom. This enhanced sediment community oxygen consumption (SCOC) could be explained by a higher respiratory activity, which can be attributed, among other factors, to benthic bacteria. However, the fluorochrome acridine orange (AO) bacterial counting technique used here does not discriminate between living, dead, active or dormant cells.

Bacterial abundance was lower after the bloom than before. One explanation for the lower bacterial abundance at station 85 is that there was a generally lower standing stock of bacteria at this station (see 2.6). A second possibility is that bacteria may have been ingested by foraminifera stimulated by the phytodetritus fall (Koho et al. 2008).

To summarize, the observed shift in relative numbers of bacteria in the sediment horizons may have been produced by an input of phytodetritus-associated bacteria, the grazing of active foraminifera on bacteria in deeper layers, or even a shift in community abundance towards the first centimeter. Nonetheless, the observed oxygen consumption at station 85 supports our hypothesis that bacteria react with enhanced (respiratory) activity to a food pulse from the water column.

Reaction of meiofauna to food input

The meiofauna individual numbers found during our case study (592.4 - 1458.3 individuals per 10 cm^2) and the dominance of the Nematoda (83.1%) are comparable to the data previously reported from about 2000 to 5000 m depth in the Southern Ocean and the Weddell Sea

(Gutzmann et al. 2004; Herman and Dahms 1992; Vanhove et al. 1995, 2004).

After the blooming event, a significantly higher percentage of meiofauna organisms was found in the first sediment centimeter than before. First, meiofauna may migrate upward in order to feed on the fresh organic material on the surface. This is supported by the concordance of the vertical shift in meiofauna distribution between the sampling in December (station 13) and January (station 85) with the pigment profiles obtained during our study. Higher pigment contents were reflected in higher relative meiofauna abundance in the first centimeter. Second, meiofauna may also concentrate in the first centimeter of the sediment in order to feed on degraded material and bacteria. The meiofauna shift towards the sediment surface in January (station 85) matched the higher relative numbers of bacteria on that sampling date. Third, meiofauna may avoid deeper sediment layers that are oxygen-depleted. The observed shift matched the changes in the oxygen vertical profile detected during our study, and reflected the steep depth gradient in oxygen concentration observed in January (station 85).

As the measured OPD at both stations was still comparable, the possibility arises that the migration toward upper sediment layers was mainly due to the enhanced availability of organic material. However, there is no information on the sensitivity of the organisms to low oxygen concentrations at our stations, and the possibility that oxygen consumption is a main factor regulating the meiofaunal migration toward upper sediment layers must be kept in mind.

At the Porcupine Abyssal Plain, Gooday et al. (1996) sampled before (April) and after a spring bloom (July). Meiofauna did not migrate toward the sediment surface after the phytodetrital pulse, and, in contrast to the foraminifera from the same samples, metazoan meiofauna did not exploit the detrital food rapidly. In our study we showed that deep-sea meiofauna indeed can move toward a newly available food source. However, it still remains unclear how the meiofauna exploits these resources. In the Cretan Sea (Mediterranean), bathyal meiofauna communities reacted with a time lag of two months to food input (Danovaro et al. 2000). There, meiofauna utilized increased bacterial biomass resulting from pulses in particulate organic matter, rather than the refractory organic material itself. Recent experimental findings from the Arctic deep sea (Guilini et al. 2010) confirm the unclear feeding situation for benthic deep-sea nematodes (compare Witte et al. 2003b). Guilini et al. (2010) explained the very limited accumulation of ^{13}C-labeled carbon during a feeding experiment by postulating a passive uptake of ^{13}C-labeled substrates by the nematodes, rather than via bacterivory. Ingels et al. (2010b) found very low uptake rates as well. However, they observed a preference for bacteria over phytoplankton in Arctic and Antarctic abyssal nematode communities in a pulse-chase experiment.

In the bathyal Sagami Bay, Shimanaga et al. (2000) detected seasonal changes in the vertical

distribution of Copepoda (high-resolution slicing steps of 2.5 mm) and suggested that they were related to the amount of chloroplast equivalents (CPE) available in the sediment. The most abundant taxon, the Nematoda, did, however, not react to this input of organic matter. These findings are in contrast to the results of this study, where the measured difference in vertical distribution of the meiofauna community was mainly due to higher relative abundances of nematodes at the sediment surface. However, we did not slice our samples in the same narrow vertical steps applied by Shimanaga et al. (2000) and therefore may not have detected the small-scale vertical migration performed by copepods that they observed. Moreover, the sediments in Sagami Bay showed an oxygenated layer between 0.8 and 2.3 cm depending on the season. This is a very thin oxygenated layer compared to the situation that we encountered in the Antarctic.

The situation in the abyss at 52°S is comparable to the findings of Franco et al. (2008) who monitored the reaction of meiofauna to phytoplankton deposition at a shallow-water station (20 m depth) with permeable sediments in the North Sea. The authors detected significant differences in the vertical distribution of nematodes in the upper 5 cm, probably as a result of an upward migration of the animals toward their food source (Franco et al. 2008). Although this study was carried out in shallow water, the sediment was described to have highly permeable sediments (redox values > 100 mV at all sediment depths; Vanaverbeke et al. 2004). Redox values of 100 mV may be expected for oxygen values of ~80 µM l^{-1} (Forster and Graf 1992). We found comparable oxygen concentrations at ~10 cm sediment depth at station 13, and ~5 cm sediment depth at station 85. Thus, nematodes in the deep sea may show the same migration reaction to enhanced food availability on the sediment surface.

Copepod reproductive effort

In contrast to foraminiferans, which can increase their metabolic activity very rapidly, there is a higher energetic expense of egg production in combination with slower rates of somatic growth in metazoans (Gooday et al. 1996). Before detectable responses in numbers of eggs and larvae appear, copepods need time to incorporate nutrients and produce eggs. Additionally, the development time of embryos of benthic copepods depends on environmental temperature, egg size, and adult size (G. Veit-Köhler and T. Brey, unpubl. data).

At 52° S the meiofauna did not react immediately with a detectable reproductive effort in terms of enhanced egg production in copepods or increasing numbers of copepod nauplius larvae, to the seasonal but highly patchy food input. This delay was expected; for instance, Mediterranean bathyal meiofauna communities showed a time lag of two months between food input and a reaction in terms of increased community density, copepod nauplii, and individual biomass of higher meiofauna taxa (Nematoda, Copepoda, Polychaeta) (Danovaro et al. 2000).

Benthic organisms and sediment community oxygen consumption (SCOC)

Witte et al. (2003a, b) reported considerable increases in SCOC shortly after artificial phytodetritus pulses in the Sognefjord (1265 m depth, OPD 16.8 mm, SCOC increased 25% from 3.6 to 4.5 mmol O_2 m^{-2} d^{-1} within 3 d) and the Porcupine Abyssal Plain (PAP, 4800 m depth, OPD 15 cm, SCOC nearly doubled from 0.44 to ~0.8 mmol O_2 m^{-2} d^{-1} within 2.5 d). Our findings are in line with this study. The initial oxygen consumption measured in our study (0.400 mmol O_2 m^{-2} d^{-1}) is comparable to the situation found at the PAP. However, after the phytoplankton bloom, SCOC nearly tripled in our case (1.102 mmol O_2 m^{-2} d^{-1}). The enhanced oxygen consumption, higher concentrations of fatty-acid degradation products in the sediment, and particularly the population shifts at the sediment surface indicate a higher bacterial and metazoan respiratory activity at the end of January. The two sites studied by Witte and co-workers contrasted in the amount of time required by the bacteria to start incorporation of the organic material. Whereas at their slope station, bacteria fed on the labeled detritus within 3 days, at the PAP deep-sea station the bacteria required 8 days to double their activity (measured indirectly through the extracellular enzyme activity). However, at both locations, members of the macrofauna were among the first benthic organisms that ingested the fresh food. Thus, bacteria were not responsible for the initial increase of SCOC in the Porcupine Abyssal Plain.

We can only speculate how long the fresh food had been available for the benthic communities at 52° S before the second sampling took place. The total time span of our case study was 52 days. We detected migration tendencies and/or shifts in abundance in the meiofauna and the bacteria. It is possible that by the end of January the reaction of the metazoan benthic organisms was triggered by a prior use of the organic material by bacteria and foraminifera, as found by Moodley et al. (2002) and Koho et al. (2008). Another explanation is that bacteria may have reacted to an initial degradation of the material by macrofauna (Witte et al. 2003b). In their study, nematodes (as representatives for the meiofauna) reacted with a time lag of 8 days. In an *ex situ* pulse-chase experiment, Moens et al. (2007) added labeled freeze-dried cyanobacteria to sediment cores from a 227 m-deep Antarctic site. They found evidence that although remineralization started immediately and rates were comparable to studies from deep-sea sites, nematodes took up the label only after a lag period of 16 days. The most elaborate experiment to test bacteria as a food source for deep-sea nematodes was recently carried out by Guilini et al. (2010). They found that although different bacterial functional groups were marked by different ^{13}C-labeled substrates, nematodes did not incorporate the label via the bacteria nor did they take up considerable amounts of the labeled substrates directly within 7 days.

The absence of measurable uptake of labeled food does not mean that organic matter had not been used. Benthic respiration by itself may play an important role when no measurable production of labeled biomass occurs (Relexans et al. 1996) or labeled material is found only in the guts of analyzed specimens (Levin et al. 1999). Bacteria (through assimilation and respiration) and foraminiferans were the key players in a short-term benthic response to an experimental input of marked diatoms in a benthic-chamber experiment at a deep-sea site in the northeast Atlantic (Moodley et al. 2002). The initial SCOC of 0.48 and 0.53 mmol O_2 m^{-2} d^{-1} in the benthic chambers doubled after the injection of labeled diatoms. However, with a rate of 45%, the major signal of benthic processing of the added carbon was recorded as respiration (recovered as CO_2). Baguley et al. (2008) reported for two deep stations (3545 and 3400 m) in the Gulf of Mexico that the meiofauna respiration of 0.5 and 0.3 mg C m^{-2} d^{-1} accounted for 8% - 13% of the entire sediment community respiration of 3.9 mg C m^{-2} d^{-1} at both stations. They estimated meiofauna respiration allometrically via biomass and abundance (mean values 875 and 635 ind. 10 cm^{-2}), which is comparable to our study (single cores 1,458.26-592.38 ind. 10 cm^{-2}). With a total sediment community respiration of 3.3 mg C m^{-2} d^{-1} the pre-bloom situation in our study is comparable to the two stations at similar depths described by Baguley et al. (2008).

We assume that the upward migration would not take place if meiofauna and especially nematodes did not feed on the freshly available food or a food source related to this input of organic matter such as bacteria, fungi, ciliates, or labile material. The settlement of the phytodetritus must have occurred ~3 to 25 days before the sampling took place (according to Witte et al. 2003b and the sinking rate of phytodetritus of 100 - 150 m d^{-1} given by Gooday 1993). This inference is supported by the strong reaction in terms of SCOC observed at station 85. As the phytodetritus was still clearly detectable on the sediment surface, forming a green flufflayer, we presume that the remineralization process was still in the initial stage. It has been assumed that meiofauna is important in diagenesis, deep-sea carbon budgets, and global biogeochemical cycles (Baguley et al. 2008). However, to date empirical data about feeding habits, food preferences, assimilation and respiration rates, and in general the significant role of deep-sea metazoan meiofauna are lacking.

CONCLUSION

Meiofauna and bacteria in the abyssal Southern Ocean reacted to the settlement of a phytoplankton bloom to the seafloor at 52° S, with enhanced respiratory activity and vertical migration (meiofauna) toward the sediment surface. The vertical distributions reflected the changing sediment situation, e.g. enhanced C-flux, increased SCOC, pigment contents,

and fatty-acid distribution. During the experiment no increase in cell numbers of bacteria was observed, which is in line with previous findings. Reproductive parameters of copepods (number of egg-carrying females and nauplius larvae) did not differ between the two sampling dates.

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Addendum 2

Characterization of the seafloor at the SYSTCO stations based on video observations and ground truthing sedimentology

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ABSTRACT

An underwater camcorder mounted on a lander frame was used to image the sedimentary conditions and the epibenthic fauna at the seafloor along the SYSTCO transect. Four stations were monitored; on the lower shelf (600 m), continental slope (2000 m), abyssal plain (5300 m), and on top of a seamount (2100 m), respectively. The sediment surface was filmed for different time lengths between three and nineteen minutes while the camera was towed over distances ranging from 11 to 187 m, resulting in areas filmed between 4 and 71 m² per station. From the gathered images, epifauna was counted and identified to the lowest possible taxonomic level. For ground truthing, two to three replicate multicorer samples per station were vertically sectioned per centimeter down to 5 cm depth and analysed for sediment granulometry. Differences in the sedimentary characteristics could be related to both water depth and topography according to the multicorer samples. The video footage from each station showed a range of different sediment surface types and widely varying epifaunal abundance, the lowest number of readily identifiable organisms being present on the abyssal plain and the highest on the lower shelf.

Keywords: Southern Ocean · Deep-sea floor · Sediment surface · Seabed video images · Epifaunal composition · Granulometry

INTRODUCTION

One of the major goals of the SYSTCO expedition (ANT XXIV-2) was to link the development and fate of a phytoplankton bloom in the Southern Ocean water column to the influx of organic carbon into the benthic deep-sea system. In previous cruises (ANDEEP I–III) to the deep Southern Ocean, sediment profiling images and surface pictures had occasionally shown thick layers of organic fluff on the abyssal and bathyal seafloors (Brandt and Hilbig 2004; Brandt and Ebbe 2007). During the planning of SYSTCO, still photography of surface and upper ca. 15 - 20 cm of the seafloor, as well as video recordings of the sediment water interface plus near-bottom water layers with the Sediment Profiling Imagery (SPI) camera system, was considered to be one of the most important methods to help describe coupling processes between the water column and the deep-sea floor.

However, due to logistic constraints, the SPI-camera system was not available for this expedition. To obtain photographic coverage of at least some of the seafloor that was sampled with other towed gear, coring devices, and landers, an underwater camcorder in a deep-sea housing (sediment surface Video Observer) was used. Even though we did not succeed in observing the arrival of the fluff layer at the single station under the Polar Front, which we

sampled twice (see Brandt et al. 2011b), observations from the other stations are presented here to provide background reference for faunal data and gain an idea of their spatial patchiness. Ground-truthing sediment granulometric data were used to verify or falsify video observations and to provide data on sediment structure down to 10 cm depth. The latter data can help in the interpretation of sedimentary characteristics in relation to the benthic fauna (i.e., horizontal layering due to bioturbation).

MATERIAL AND METHODS

Four locations were successfully sampled with the underwater camcorder (Table 1). They were located on the continental slope in the Lazarev Sea, the Weddell Sea abyssal plain, the seamount Maud Rise, and on the lower shelf near the northern pier of Neumayer Station. The aim was to visualize and document all stations that were sampled for benthic faunal studies. Due to misinterpretations of the conductivity of the coax communication cable at the first station (PS71/13-20) the first deployment of the camera system was unsuccessful. The station was revisited, but the camera was not deployed because of bad weather. The remaining four deployments (PS71/17-15, 33-17, 39-9, and 48-2) were successful (for station locations see the map in Brandt et al. 2011b).

The multicorer failed at sampling the most shallow shelf station (PS71/48) because plexicore tubes broke due to the stony character of the sediment. The single station at 52°S was successfully sampled and resampled with the multicorer, and the granulometric data are included here.

As an alternative for the unavailable SPI camera system that had been used during previous expeditions to the deep Southern Ocean (Brandt and Hilbig 2004, Brandt and Ebbe 2007), we used an electronically modified digital video camcorder from the German Center for Marine Biodiversity Research (DZMB) to document the sediment surface. The underwater camcorder (model: Nautilus Marine Service with camcorder PANASONIC NV-GS400EG) was equipped with two 300W spotlights (Deep-Sea Multi-SeaLife, P/N 710041601). All parts were installed in a 2 m high, pyramidal Lander-frame (DOCLT) (Fig. 1A and B). Additionally, two 100 kg of lead were attached to the frame to increase the weight. Using a coax communication cable, the underwater camcorder was powered up with a power supply unit to provide 450 - 550 V with 1.1 - 1.4 A. The underwater camcorder filmed at 80 cm above the sediment surface, an area of approximately 85 x 45 cm^2. The film was saved on a 6.35 mm digital videotape, in 625 lines PAL, PCM-Digital-Take record system with 16 Bit (48 kHz/2 canal) audio quality. The tape recorded a time of 60 min.

Table 1. Protocol of camera deployments during the expedition SYSTCO.

Station and Area	Date	Time	Lat	Long	Event	Usable for Observations
PS71/17-15	12/22/2007	0:39	70°04'S	003°23'W	Begin Station	
		0:42			on bottom	sequence 8
Lazarev Sea		0:44			off bottom	
		0:46			on bottom	sequence 9
		0:49			off bottom	
		0:51			on bottom	sequence 10
		0:54			off bottom	
		0:57			on bottom	sequence 11
		0:59			off bottom	to surface
PS71/33-17	12/31/2007	0:02	62°00'S	002°59'W	Begin station	
		0:03			on bottom	sequence 1
Central WS		0:14			off bottom	
		0:20			on bottom	sequence 2
		1:00			off bottom	to surface
PS71/39-9	01/03/2008	0:02	64°28'S	002°53'W	Begin station	
		0:03			on bottom	sequence 1
Maud Rise		0:06			off bottom	
		0:07			on bottom	sequence 1.1
		0:08			off bottom	
		0:10			on bottom	sequence 2
		0:15			off bottom	
		0:17			on bottom	sequence 3
		0:21			off bottom	
		0:26			on bottom	sequence 4
		0:30			off bottom	
		0:34			on bottom	sequence 5
		0:38			off bottom	
		0:43			on bottom	sequence 6
		0:46			off bottom	to surface
PS71/48-2	01/12/2008	0:02	70°23'S	008°19'W	Begin station	
		0:03			on bottom	sequence 1
Northern Pier		0:05			off bottom	
		0:12			on bottom	sequence 2
		0:15			off bottom	
		0:21			on bottom	sequence 3
		0:24			off bottom	
		0:29			on bottom	sequence 4
		0:32			off bottom	
		0:36			on bottom	sequence 5
		0:41			end of tape	

Figure 1. Underwater camcorder mounted on lander frame. A. Setup on deck. B. Detail of deep-sea camera housing, cables and spotlights (arrows).

The lander frame with the pressure chamber, including the underwater camcorder, was lowered to the sea bottom and positioned on the ground for 3 min, to allow a clear view of the sediment surface. Subsequently the lander frame was heaved 10 m and followed the drift of the ship for 2 min during which no useable images were recorded. Then a new cycle was started, and the procedure was repeated for 1 h. At the end of the station the underwater camcorder returned to the surface.

Four locations were successfully sampled with the underwater camcorder (Table 1). They were located on the continental slope in the Lazarev Sea, the Weddell Sea abyssal plain, the seamount Maud Rise, and on the lower shelf near the northern pier of Neumayer Station.

The video footage was viewed on-board and observations were entered immediately onto a spreadsheet recording data every second. Analysis was purely qualitative even though we estimated the area filmed, the sediment type, and the surface and shell coverage, and counted readily visible macrofauna on and near the bottom to get an impression of the differences with depth, bottom topography, and geographic position.

Sediment grain size distribution was measured using multicorer samples taken at the same locations as the video footage. From two to three indepedent multicorer samples (9.4 cm

internal diameter) subsamples were taken with 2 cm diameter syringes. The sediment in the syringes was sliced into 1 cm fractions down to 5 cm and immediately frozen at -20 °C. In the laboratory, grain size distribution was measured using a Coulter Counter LS 100TM Particle Size Analyser. The 0.06 - 1000 mm sediment fractions were expressed in volume percentage (vol %) and classified according to Wentworth (1922).

RESULTS

Continental slope, Lazarev Sea (Station PS71/17)

The station was located in approximately 2000 m water depth. Generally the seafloor consisted of mud with about 5% - 20% stone coverage (Fig. 5A). The fauna was relatively rich, with 128 individuals seen on the bottom during about 13 min, covering a distance of about 190 m and a monitored area of 71.5 m^2 (Table 2). Among the observed epifaunal taxa that were seen more than 5 times were a deep-sea shrimp (*Nematocarcinus longirostris*), holothuroids with at least two species, the octocoral *Umbellula* sp., and ophiuroids (Table 3). Isopods, amphipods, sponges, polychaetes, and others were only seen once or in very few instances. No **fish** were encountered.

The sediment grain size analysis revealed relatively coarse sediments with the highest percentage of sand among the sampled stations, besides approximately 49% - 64% of silt (Table 4). Percentages of both silt and clay were the lowest in the top 2 cm and the highest at 4 - 5 cm depth (Fig. 2A), indicating lamination.

Table 2. Locations, sampling details, sediment characteristics and faunal data for each underwater camcorder deployment.

Area	Depth (m)	Sediment/ Surface Coverage	Stones/ Surface Coverage	Shell Fragments/ Surface Coverage	Visible Macrofauna (>5cm) total	Vertebrata total	Pelagic total	Usable Time (min)	Distance photographed (m)	Area photographed (m^2)
Lazarev Sea	1960 - 2044	soft 80% - 95%	5% - 20%		128	0	0	12.9	187	71.5
Central Weddel Sea	5349 - 5344	very soft 100%			1	0	0	3.0	11	4.2
Maud Rise	2117 - 2120	coarse ~98%	1% - 2%		18	2	8	12.8	79	30.2
near Northern Pier	594 - 616	compacted ~55%	~40%	~5%	291	7	0	18.9	151	57.8

Table 3. Macrofauna observed at three stations on the continental slope, Lazarev Sea (Sta. 17), Maud Rise (Sta. 39), and on the lower shelf near Northern Pier (Sta. 48). The single organism seen on the Weddell Sea abyssal plain (Sta. 33) is not listed.

Sta. 17-15	*Nematocarcinus longirostris* (Decapoda)	38
	pink aff. *Bathyplotes* (Holothuroidea)	28
	aff. *Umbellula* sp. (Octocorallia)	22
	Ophiuroidea	12
	Holothuroidea "sandy"	8
	Actiniaria	3
	Amphipoda	3
	shrimp	2
	Evertebrata indet.	2
	Porifera	1
	Jellyfish	1
	Polychaeta	1
	Isopoda	1
	Munnopsidae sp. (Isopoda)	1
	Pycnogonida	1
	Echinoidea	1
	aff. *Porania* sp. (Asteroidea)	1
	aff. Ascidiacea	1
	Brachiopoda	1
	Total	128
Sta. 39-9	Holothuroidea	7
	Chaetognatha	3
	Actiniaria	2
	Gigantocypris sp. (Ostracoda)	2
	Echinoidea	2
	Evertebrata indet.	2
	Pisces indet.	2
	Pycnogonida	1
	Total	21
Sta. 48-2	Crinoidea	177
	Porifera	29
	Asteroidea	28
	aff. Ascidiacea	12
	Actiniaria	10
	Ophiuroidea	9
	Pisces indet.	7
	Bryozoa	7
	Polychaeta	6
	Hydrozoa	4
	Chaetognatha	1
	Sea Pen	1
	Total	291

Table 4. Sediment grain size composition at Sta. 17, 33, and 39, average of two or three multicorer samples, in 1-cm depth intervals.

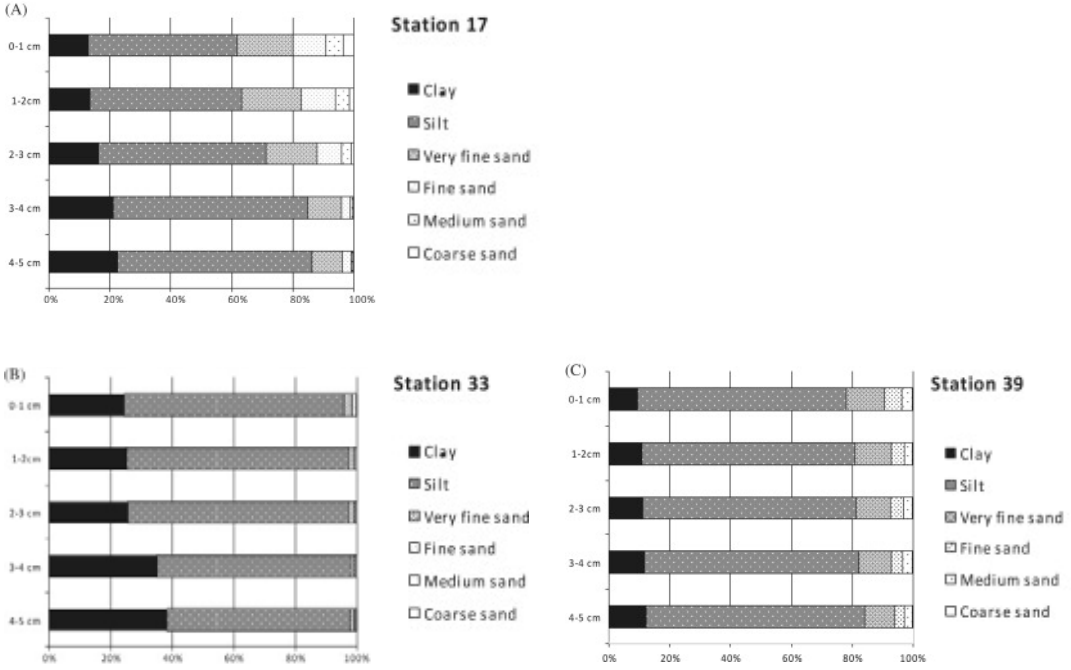
Depth fraction (cm)	Percent clay	Percent silt	Percent very fine sand	Percent fine sand	Percent medium sand	Percent coarse sand
Station 17 (2 MUCs)						
0 - 1	12.646	48.742	18.349	10.681	5.583	3.430
1 - 2	13.346	49.990	19.603	11.121	4.631	1.310
2 – 3	16.272	55.082	16.738	7.987	3.043	0.879
3 - 4	21.008	63.901	11.079	2.988	0.821	0.204
4 - 5	22.466	63.798	10.076	2.694	0.711	0.256
Station 33 (2 MUCs)						
0 - 1	24.491	71.221	2.326	0.478	1.130	0.354
1 - 2	25.159	72.037	1.797	0.453	0.486	0.069
2 - 3	25.477	71.787	1.866	0.274	0.507	0.090
3 - 4	35.063	62.915	1.251	0.190	0.518	0.063
4 - 5	38.314	59.421	1.273	0.228	0.570	0.194
Station 39 (3 MUCs)						
0 - 1	9.303	68.751	12.657	5.539	3.542	0.208
1 - 2	10.751	70.234	11.883	4.179	2.788	0.165
2 - 3	11.150	69.988	11.461	4.266	2.886	0.249
3 - 4	11.586	70.405	10.871	3.802	2.915	0.420
4 - 5	12.354	71.681	9.987	3.030	2.531	0.417

Weddell Sea abyssal plain (Station PS71/33)

This station was the deepest, located on the abyssal plain of the central Weddell Sea at about 5300 m depth. According to the surface photographs and the presence of large clouds of fine sediment in the water column caused by accidental contact of the lander frame with the seafloor that were settling very slowly, the bottom consisted of very soft mud; stones were however not seen (Fig. 5B). Epifauna was extremely rare, and only a single anemone was observed. This result was in part due to a long time period of more than 40 min during which the camera did not move at all, resulting in a monitored area of only 4.2 m^2; however, motile organisms crawling on the bottom or swimming in the near-bottom water column were not seen either, indicating a generally low organism density of organisms.

The sediment grain size analysis showed similar results. The sediment consisted almost entirely of 95.7% - 97.7% silt and clay (Table 4), with a rather pronounced fining with depth resulting in a roughly 1.5-fold increase in clay content with depth in the sediment (Fig. 2B).

Figure 2. Average sediment grain size distributions. A, Station 17 (Lazarev Sea). B, Station 33 (Weddell Sea abyssal plain). C, Station 39 (Maud Rise).



Maud Rise (Station PS71/39)

The video station was located near the top of the seamount Maud Rise, about 2100 m deep (Table 2). The surface of the seafloor looked coarse, about 1% - 2% of the 30.2 m^2 of monitored surface was covered with stones (Fig. 5C). The abundance of visible fauna was low, comparable to that on the abyssal plain. The only organisms seen more often than once or twice were holothuroids and near-bottom chaetognaths (Table 3). One of the more remarkable sights was the observation of two *Gigantocypris* spp. Mega- and macrofauna samples from the Agassiz trawl and epibenthic sled contained similar low abundances of organisms.

The sediment grain size analysis indicated that the sediment at Maud Risee is vertically more homogeneous than at the other stations, with a relative contribution of silt, clay, and very fine to coarser sands that was similar down to 5 cm sediment depth (Fig. 2C).

Lower Shelf near Northern Pier (Station PS71/48)

The station was located near the Northern Pier of the Neumayer Station in approximately 600 m depth on the lower shelf (Table 2). Sediments were compacted, possibly by iceberg scouring, and about 45% of the 57.8 m^2 of monitored surface was covered by stones (Fig. 5D). Epifaunal density was distinctly higher on this shelf site than on the lower slope and abyssal sites, as might be expected (Table 3). The most abundant epifauna observed were crinoids and sponges, but there were also many more fish than at the other stations. The distribution of epifauna was patchy, especially with regard to the crinoids.

Sediment grain size data are not available as no multicorer samples could be taken in the coarse and compacted sediment.

Southern Polar Front (Stations PS71/13 and PS71/85)

Both samplings in the beginning of December and at the end of January, respectively, produced sediment cores that consisted of more than 77% silt and almost no sand down to 5 cm sediment depth (Table 5, Fig. 4). No difference was evident between the two sets of samples, one taken during and the other after the algal bloom (Herrmann and Bathmann 2010).

Figure 3. Synopsis of average sediment grain size distributions to show bathymetric and topographical influences. Sta. 39: top of the seamount Maud Rise, with relatively high sand content and almost no stratification with sediment depth; Sta. 33: Weddell Sea abyssal plain, very fine sediments, distinct stratification (increasing clay content) with sediment depth; Sta. 17: continental slope, Lazarev Sea, mixed sediment with pronounced stratification indicated by decreasing silt/clay fraction with sediment depth.

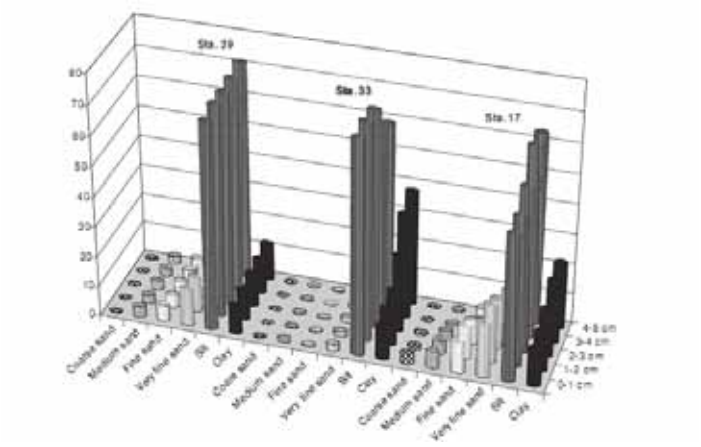


Figure 4. Synopsis of sediment grain size distribution at Station 13/Station 85 (revisit after settling of algal bloom).

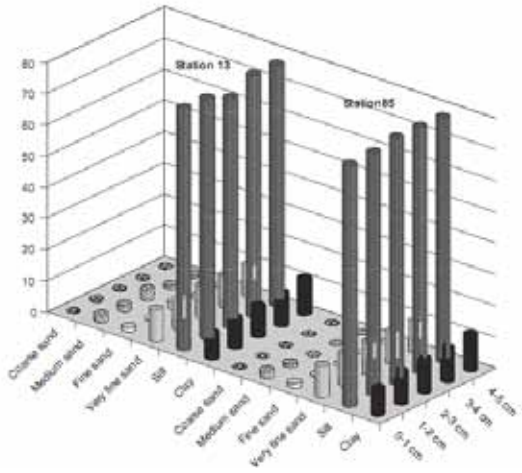
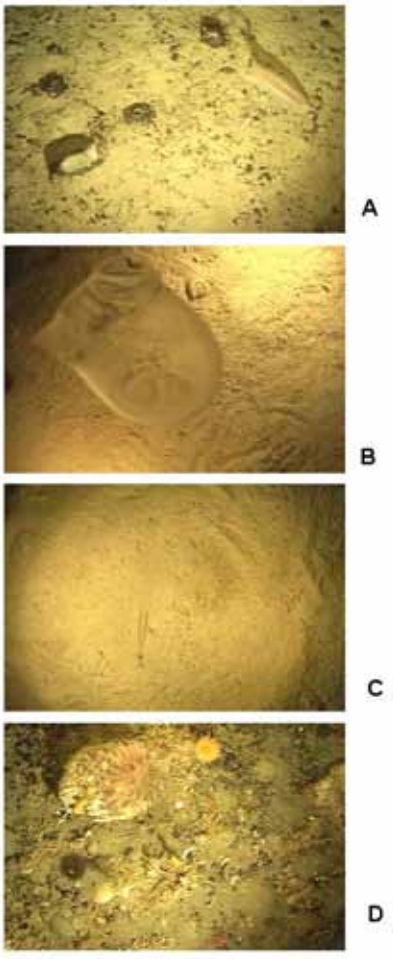


Figure 5. Single frames from video tapes. A, continental slope, Lazarev Sea, with several scattered small and larger stones, sea cucumber on the upper right. B, Weddell Sea abyssal plain, fine sediment with Lebensspuren left by sea cucumbers. The water drop in the center of the image was caused by a partial crack of the glass dome protecting the camera lens. The water intrusion stopped after the initial cracking, and the camera came back to the surface intact. C, Maud Rise, surface appearing evenly fine, possibly because of a very thin upper layer of clay carpeting the sandier sediments. A large chaetognath apparently living near the sediment is swimming through the center of the image. D, lower shelf near Northern Pier, very heterogeneous bottom with stones and a rich epifauna characteristic of the shelf.



DISCUSSION

The use of camera systems to characterize the benthic environment has proven quite useful as large areas can be covered in a relatively short time once the camera reaches the bottom (Howe et al. 2007). Sediment Profiling Imagery (SPI) has the greatest advantage to document sedimentary characteristics not only from the surface, but also down to about 15 - 20 cm depth, providing the dimension of time. Unfortunately, no SPI system was available for this expedition, forcing us to rely on a relatively simple system of a video camera mounted on a pyramidal frame that could only deliver impressions of the general surface structure of most of the stations that were sampled with coring devices and towed gear. The results cannot be quantified as there are only rough estimates on the area photographed; however, in connection with sediment grain size analysis based on multicorer samples taken at the same locations, an informative overview of the different environments can be given and some trends can be seen.

Not unexpectedly, there was a depth related trend in the composition of the sediment (Fig. 3). Sediment texture as observed by the camera changed from compacted soft bottom of undetermined grain size mixed with many stones and shell fragments on the lower shelf near the Northern Pier to soft mud mixed with a lesser amount of stones on the continental slope to extremely fine mud on the abyssal plain.

The most distinct difference, however, was related to topography, namely between sediments on the Lazarev Sea slope and those on top of the seamount Maud Rise (both at about 2000 m depth). The seafloor at the top of Maud Rise, although with very few stones, appeared coarse on the video images rather than fine and muddy. As current speed was found to be relatively high at 1.25 - 2.66 m/s over the top of the seamount, finer particles could have been washed out. Nonetheless, grain size analysis based on multicorer samples showed clear differences only in the content of silt, which was much higher in the top centimeters of sediments from Maud Rise than on the Lazarev Sea slope; clay percentage, on the other hand, was slightly lower in Maud Rise samples than in continental slope samples. As roughly two-thirds of all macrofaunal organisms caught with the epibenthic sledge on Maud Rise were bivalves and gastropods (Brandt et al. 2011a), it is also possible that their shells or possibly fecal pellets gave a coarser optical appearance of the surface on video records.

The presence of horizontal layering in sediments from the continental slope can possibly be related to the presence of turbidites, leading to increase in silt and clay and decrease in sand with sediment depth. Preliminary on-board examination of samples taken at Sta. 17 (continental slope of the Lazarev Sea) with the Agassiz trawl and epibenthic sledge revealed a rich and abundant infauna consisting of isopods and amphipods as well as polychaetes, all

of which may be involved in reworking of the sediments. The polychaetes included specimens belonging to the Spionidae and Maldanidae, both known for their bioturbating activities.

The benthic infaunal community on Maud Rise turned out to be quite different from all other samples both in composition and density (Brandt et al. 2011a; Wilmsen and Schüller 2011), resulting in less bioturbation except very close to the sediment water interface, increased filter feeding, and almost no horizontal stratification of the upper 5 cm of sediment.

On the abyssal plain, stratification was observed as well. The bioturbating organisms potentially causing this pattern, however, were different from the slope. Megafauna catches (macroepifauna was not sampled at this station) consisted mainly of holothurians (Janussen et al. 2010), while the remaining megafauna was depauperate. As no large animals were observed with the camera, distribution of the holothurians may be patchy.

The station under the subpolar front did not change with regard to the granulometry within the seven weeks between the two sampling events (Fig. 4). During that time, a plankton bloom had descended to the seafloor as marine snow. The arrival of organic matter on the deep-sea floor was detected by elevated biological activity (Sauter and Sachs 2010). As the sediments were quite homogeneous down to several centimeters, even increased bioturbation would not have resulted in readily visible changes in sediment texture. This result was therefore not unexpected, although it is possible that biogenic changes may have occurred with a time lag of several weeks.

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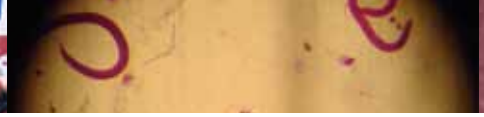


This PhD study provides a detailed analysis of structural and functional aspects of the ecology of free-living nematodes in different deep-sea ecosystems. The role of food and oceanographic conditions was studied by means of field observations and experiments. Performed experiments are innovative and revealed new insights in deep-sea nematode feeding behavior, rates and mechanisms of colonisation, and the role of small-scale disturbances in establishing and maintaining nematode diversity. Field observations from both photosynthetic and chemosynthetic driven ecosystems further revealed the role of both bacteria and phytodetritus in the diet of deep-sea nematodes and provided insights in the influence of oceanographic conditions on both the structure and function of nematode communities. Obtained data rendered post-hoc explanations from which new testable ideas were formulated. Considering the great importance of understanding dynamics of food consumption in different deep-sea ecosystems in order to understand the global carbon cycle and the impact of potential future climate changes, this PhD thesis may serve as pioneer work on which future research can build.