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

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ABSTRACT

Sediments sampled from the Galicia Bank seamount and the adjacent slope (northeast Atlantic), and from a western Mediterranean slope site, were injected onboard with ^{13}C-enriched dissolved organic matter (DOM) to evaluate nematode feeding strategies and the fate of DOM carbon in different benthic environments. We hypothesized that nematode ^{13}C label assimilation resulted from either direct DOM uptake or feeding on ^{13}C labeled bacteria. Slope sediments were injected with glucose ("simple" DOM) or "complex" diatom-derived DOM to investigate the influence of DOM composition on carbon assimilation.

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

Our results did not indicate substantial differences in carbon processing related to the complexity of the DOM substrate. The quantity of processed carbon (5–42% of added DOM) was determined by the bacteria, and was primarily respired. The bulk of the added ^{13}C-DOM was not ingested by the benthic biota under study, and a considerable fraction was possibly adsorbed onto the sediment grains.

1. Introduction

With increasing water depth, there is a higher contribution of the metazoan meiofauna, dominated by the nematodes, to total community biomass and abundance (Rex et al., 2006; Wei et al., 2010). In coastal food webs, nematodes are believed to be important because of their high metabolic and reproductive rates and intermediate trophic position (between bacteria/detritus/microalgae and macrofauna/megafauna), in conjunction with their ubiquity and high standing stock (Giere, 2009). Whether this also applies to deep-sea species is unknown, since information on their life history strategies is entirely lacking. Feeding strategies of nematodes are generally inferred from the morphology of their buccal cavity (Wieser, 1953). However, a disadvantage of Wieser's trophic classification scheme is that it does not take into account feeding selectivity or flexibility (Moens et al., 2004; Olafsson et al., 1999). Moreover, empirical verification of the classification is limited to a few observational studies on shallow-water nematodes (Jensen, 1987; Moens and Vincx, 1997), whilst the applicability of the scheme for deep-sea species is not proven.

According to Wieser's classification, the dominant nematode genera in the deep sea are deposit (feeding types 1A+1B; e.g. *Monhystrella* and *Halalaimus*) or epistrate (2A; e.g. *Acantholaimus*) feeders, which can be regarded as potential bacterivores (Jensen, 1988; Soetaert and Heip, 1995; Tietjen, 1984). In shallow-water environments, deposit feeding nematodes feed largely on bacteria and protozoans, while epistrate feeders feed on diatoms (Moens and Vincx, 1997). Nonetheless, epistrate feeding nematodes are often abundant in deep-sea sediments despite the lack of fresh and intact diatom cells, so it seems plausible that in this

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environment, they use their small teeth to scrape off microbial coatings from sediment particles or mucus threads (Moens and Vincx, 1997). The quantitative significance of bacteria to the diet of predatory/scavenging nematodes (feeding type 2B) is considered negligible, since (1) Moens et al. (1999b) detected no to very limited uptake of bacterial cells by intertidal predatory nematodes, (2) Fonseca and Gallucci (2008) found that the gut content of deep-sea predatory nematodes often contained nematode remains and (3) during nematode identification, we observed several predatory specimens in the process of swallowing another nematode.

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

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

The injection of stable isotope labeled dissolved organic matter (DOM) into marine sediments allows the quantification of carbon flows through nematodes and bacteria (Boschker and Middelburg, 2002; Guilini et al., 2010; van Oevelen et al., 2006a). Benthic heterotrophic bacteria assimilate label by consuming DOM directly (Carlson, 2002), whereas nematodes can incorporate ^{13}C-DOM starting from two potential end-members: the DOM itself (direct DOM uptake was demonstrated for shallow-water nematodes by Chia and Warwick, 1969; Montagna, 1984; Riemann et al., 1990) or labeled bacteria. Time-series experiments elucidate the temporal dynamics of the isotope label, which can help distinguish between these two uptake pathways (Guilini et al., 2010; van Oevelen et al., 2006b). Immediate and transient ^{13}C-labeling may point to direct DOM consumption, whereas delayed, and longer-lasting ^{13}C enrichment may indicate bacterivory. Important benefits of this experimental approach are (1) the labelling of the natural, in situ benthic bacterial community (opposed to the addition of freeze-dried, "alien" bacteria) and (2) the occurrence of enriched bacteria throughout the sediment column (vs enriched bacteria only present at the sediment–water interface).

We conducted an on-board time-series experiment (1, 7 and 14 days) with ^{13}C-enriched glucose on sediments from the Galicia Bank seamount (1200 m, northeast Atlantic) to identify nematode feeding strategies. Sediment cores were sliced horizontally to assess sediment depth-dependent feeding behaviour. In addition, we conducted on-board experiments with sediments from two 1900-m-deep slope sites in the northeast Atlantic and the Mediterranean, for which we quantified isotope enrichment at day 7 only. The results of all experiments were used to quantify the potential importance of bacteria and DOM to the nematode diet under either a strictly bacterivorous or a strict DOM feeding strategy. For each hypothetical feeding strategy, we (a) compared the ^{13}C enrichment of the nematodes with that of both candidate food sources and (b) calculated the contribution of DOM and bacterial carbon uptake to nematode carbon demands inferred from respiration rates and nematode growth efficiencies. These empirical results were also compared with the findings from the feeding type classification *sensu* Wieser (1953). At the slope sites, we provided either ^{13}C-glucose as a "simple" DOM substrate or "complex" DOM extracted from ^{13}C-enriched diatoms (hereafter referred to as diatom DOM). Since nitrogen is a possibly limiting nutrient for deep-sea deposit feeders (such as the bulk of deep-sea nematodes) (Jumars et al., 1990), we expected that nematodes would prefer diatom DOM over glucose. Besides nematode feeding strategies, the experimental data were used to quantify the potential fate of "simple" and "complex" DOM carbon.

2. Materials and methods

2.1. Study sites and sampling strategy

We collected sediment samples in 2008 at the north-western Iberian margin, northeast Atlantic, at 1200 (RV *Belgica*) and 1900 m water depth (RV *Pelagia*), and in 2009 at 1900 m depth in the Algero-Provençal Sea, western Mediterranean (RV *Pelagia*) (Fig. 1, Table 1). These two regions were selected within the multidisciplinary EuroDEEP-funded research project called "BIOdiversity and Ecosystem FUNctioning in southern European deep-sea environments: from viruses to megafauna" (BIOFUN). The north-western Iberian margin is characterized by relatively high primary productivity owing to intense, wind-driven upwelling during spring and summer (220 g C m^{-2} yr^{-1}) (Joint et al., 2002; McClain et al., 1986). In comparison, the Algero-Provençal Sea has lower surface primary productivity (~153 g C m^{-2} yr^{-1}; Bosc et al., 2004) and the high water temperature (13 °C) accelerates the degradation of surface-produced OM sinking to the seabed, such that the OM pool available to the Mediterranean benthos is presumed to be more refractory than that at the northeast Atlantic sites. The 1200 m northeast Atlantic site was situated on the Galicia Bank, whereas the 1900 m northeast Atlantic (GB1900) and the 1900 m Mediterranean (WMed1900) sites were located on the slope (Fig. 1B and C). The summit of the Galicia Bank experiences strong intertidal currents (median current speed: 8 cm s^{-1}, up to 30 cm s^{-1}; Duineveld et al., 2004) winnowing the fine sediments and leaving behind a thick cover of foraminiferal ooze with high median grain size (Duineveld et al., 2004; Flach et al., 2002; van Weering et al., 2002). In comparison, the

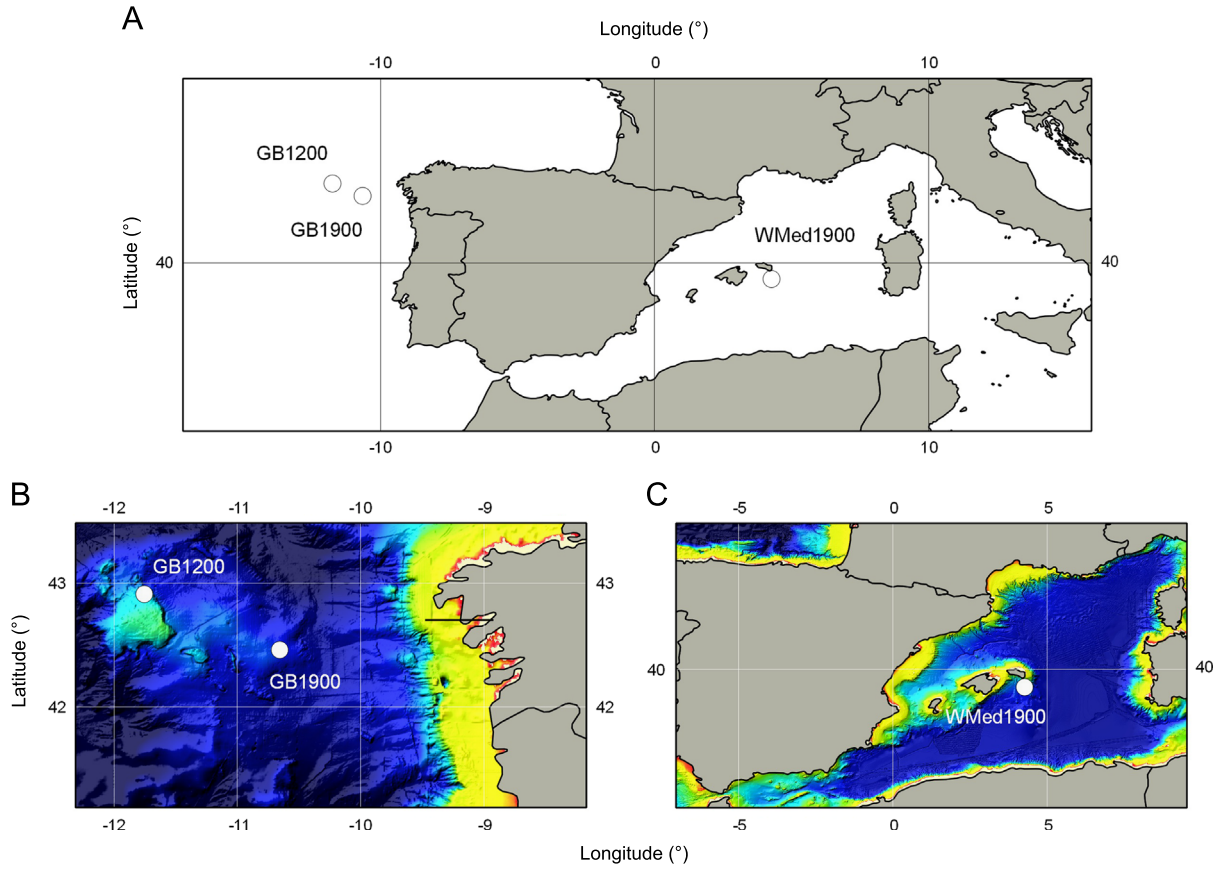

Fig.1. (A) Overview map of sites GB1200, GB1900 and WMed1900. Bathymetry of (B) the northeast Atlantic and (C) the Mediterranean sites. GEBCO bathymetric data were downloaded from EMODnet on 16/03/2012.

Table 1
Details of the northeast Atlantic and Mediterranean sites. Temp.: bottom-water temperature. For the northeast Atlantic sites, a water depth range is given over the three replicate deployments; for site WMed1900, the position of only one station was noted. Latitude and longitude are expressed in decimal degrees. Pore-water conc.= pore-water concentration.

Site	**GB1200 Northeast Atlantic**	**GB1900 Northeast Atlantic**	**WMed1900 Western Mediterranean**
Water depth (m)	1139–1141	1770–1896	1582
Date sampled	June 2008	Oct–Sept 2008	Nov 2009
Lat	42.9121	42.4607	39.4167
Long	−11.7522	−10.6547	4.2667
Temp. (°C)	10	4	13
Horizontal slicing			
Control samples	0–1, 1–2, 2–3, 3–4 cm	0–1 cm, 1–2 cm	0–2 cm
Experimental samples	0–1, 1–2, 2–3, 3–4 cm	0–2 cm	0–2 cm
Storage			
Control samples	Frozen	Frozen	Formalin
Experimental samples	Frozen	Frozen	Formalin
^{13}C labeled DOM substrates			
Type	Glucose	Glucose and diatom DOM	Glucose and diatom DOM
Amount (mg C m^{-2})	60	100	100
Pore-water conc. (μM)	135.1	568.5	568.5
Injection mode	Syringe gradually emptied over 5 cm sediment depth	Syringe entirely emptied at 1 cm sediment depth	Syringe entirely emptied at 1 cm sediment depth

northeast Atlantic (average current speed: 4.1 cm s^{-1}; R. Jeffreys, pers. obs.) and Mediterranean (average current speed: 3.8 cm s^{-1}; Jeffreys et al., 2011) slope sites were more tranquil.

At all sites, we collected three replicate field samples for grain-size and biogeochemical analysis (total organic carbon, total nitrogen and total organic matter content) and another three replicates for nematode community analysis. In the northeast Atlantic, we obtained field samples by sub-sampling cylindrical NIOZ-type box cores with polycarbonate multicorer tubes of 9.5 (surface area: 70.88 cm²) and 10 cm (surface area: 78.54 cm²)

internal diameter (i.d.) at the 1900 and 1200 m site, respectively. In the Mediterranean, multicorer samples (i.d. 6 cm; surface area: 28.35 cm²) were collected. Samples for granulometric and biogeochemical analysis were stored frozen, whereas sediment samples for nematode community analysis were fixed in seawater-buffered 4% formalin. In the Mediterranean, each of the three replicate samples for community analysis comprised two pooled cores amounting to a sample surface area of 56.5 cm². All field samples were sliced per cm down to 4 cm sediment depth.

2.2. Field samples

2.2.1. Grain-size and biogeochemical analysis

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

2.2.2. Nematode community analysis

We washed the formalin-fixed samples over a 32 μm mesh sieve and extracted the meiofauna from the sediment by Ludox centrifugation, followed by staining with Rose Bengal (Heip et al., 1985). All nematodes in each sample were counted and converted to densities per 10 cm². Where possible, ca. 100 nematodes were hand-picked from each sample and identified to genus level. Since it was difficult to distinguish between *Microlaimus* and *Aponema*, specimens belonging to one of these genera were allocated to a *Microlaimus/Aponema* genus complex. Nematodes were grouped into four feeding types on the basis of buccal morphology *sensu* Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate feeders (2A), and scavengers/predators (2B). In this study, feeding types 1A, 1B and 2A were considered potential bacterivores.

Because total carbon uptake is determined by consumer biomass (see Section 2.3.3.), we assessed genus and trophic composition based on the relative biomass of genera and feeding types. Therefore, we measured length (L, μm) and maximal width (W, μm) for each nematode to estimate individual wet weight (WW) using Andrassy's (1956) formula, adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm^{-3}; μg $WW = L \times W^2/1500{,}000$). Individual biomass ($B$) in terms of μg C ind^{-1} was then calculated as 12.4% of WW (Jensen, 1984), averaged per genus/feeding type and multiplied by relative genus/feeding type densities to obtain genus/feeding type biomass relative to total nematode biomass (i.e. "relative genus/feeding type biomass"). Relative genus and feeding type densities in the 0–2 cm sediment layer for all three sites were obtained by summing the genus counts for the 0–1 and 1–2 cm layers, taking into account total nematode abundances in each layer.

2.2.3. Nematode respiration rates

Total nematode biomass (μg C m^{-2}) was calculated as the product of individual biomass (B, μg C ind^{-1}; calculated from the isotope ratio mass spectrometer (IRMS) readings divided by the number of nematodes in the sample) and average density (determined from the field samples; ind. m^{-2}). Biomass-specific nematode respiration rates (R, μg C μg C^{-1} d^{-1}) were estimated using the formula of de Bovée et al. (1990), divided by B:

$$R = \frac{0.0449 \times B^{0.8554} \times \exp^{\ln Q10/10(T-20)}}{B} \quad (1)$$

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

2.3. Isotope enrichment experiments

2.3.1. ^{13}C-labeled substrates

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

2.3.2. Experimental setup

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

2.3.2.2. Experiments with ^{13}C-diatom DOM and ^{13}C-glucose at the slope sites. Experimental cores (i.d. 6 cm) from the slope sites were incubated for 7 days in the dark at in situ bottom-water temperature (see Table 1). The overlying bottom water in the cores was aerated with aquarium pumps. We amended the cores with DOM carbon equivalent to an addition of ca. 100 mg C m^{-2}, resulting in a pore-water concentration of 568.5 μM in the 0–2 cm sediment layer. Injection of the ^{13}C-labeled substrates proceeded

in the same manner as for the time-series experiment at GB1200, except that here, we injected the entire amount of DOM at 1 cm sediment depth. Per treatment (diatom DOM or glucose), there were two replicate cores. After 7 days, the top 2 cm of each core was removed and gently homogenized. From each 0–2 cm layer, 10 ml was analyzed for $\delta^{13}C$ of bacteria-specific PLFAs, while the remaining sediment was stored for nematode $\delta^{13}C$ analysis. Nematode $\delta^{13}C$ samples from GB1900 were stored at −20 °C, whereas those from WMed1900 were fixed in 4% formalin.

2.3.3. Isotope and PLFA analysis

Sediment samples for carbon isotope analysis (only for seamount site GB1200) were freeze-dried, ground with mortar and pestle, and acidified with dilute HCl to remove carbonates. Samples for nematode isotope composition (consisting of minimal 2.5 μg C) were obtained by hand-picking 100–150 nematodes per sample (after elutriation with Ludox) and transferring them to a few drops of Milli-*Q* water in pre-combusted (450 °C for 3 h) aluminium cups. Next, cups were dried overnight at 60 °C, closed and stored in a desiccator until analysis. Organic carbon content and isotope ratios of sediment and nematode samples were measured using a Thermo Flash EA 1112 elemental analyser coupled with a Thermo Delta V Advantage Isotope ratio mass spectrometer.

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

Carbon isotope values were expressed in the δ notation relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C\ (‰)=[(R_{sample}/R_{VPDB})-1]\times10^3$, where R_{sample} is ^{13}C:^{12}C of the sample and R_{VPDB} is 0.0111,802 (Post 2002). DOM carbon uptake was evaluated in terms of relative ^{13}C enrichment ($\Delta\delta^{13}C\ [‰]=\delta^{13}C_{sample}-\delta^{13}C_{background}$), for the seamount site, and total DOM carbon uptake (μg C m^{-2}), for all sites. Total DOM carbon ($^{12}C+^{13}C$) uptake was calculated as ^{13}C uptake divided by the fractional abundance of ^{13}C ($^{13}F=^{13}C/(^{13}C+^{12}C)$) in the labeled substrates. ^{13}C uptake was computed as the product of excess ^{13}C (E) and total carbon stock of the different compartments (bacteria, nematodes and sediments). E is the difference in the fractional abundance of ^{13}C between control ($^{13}F_{background}$) and enriched ($^{13}F_{sample}$) samples: $E=^{13}F_{sample}-^{13}F_{background}$. Total DOM carbon uptake by the nematodes was assumed to primarily reflect carbon assimilation, since the incubation periods (1, 7 or 14 days) largely exceeded previously reported defecation intervals of coastal nematodes (in the order of minutes, see also Moens et al., 1999b). To enable comparison with the slope sites, DOM carbon assimilation at the seamount site (GB1200) was recalculated for the 0–2 cm layer sampled at day 7. This was done by taking the average $\delta^{13}C$ values of the bacterial PLFAs and nematodes over the 0–1 and 1–2 cm layer, weighted by their respective concentrations. For the slope sites, a difference in assimilation rates between glucose and diatom DOM of at least one order of magnitude was considered large enough to indicate differential assimilation.

2.3.4. Nematode feeding strategies

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

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

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

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

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

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

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

In the second approach, we investigated if nematode assimilation of either food sources was sufficient to meet their carbon requirements. To this end, we compared the observed assimilation rates with those required to maintain biomass-dependent respiration rates (R) under a minimal nematode net growth efficiency (NGE) of 0.6 (indicated as assimilation/carbon demand) (van Oevelen et al., 2006c). The latter were regarded as the theoretical carbon demand of the nematodes, and were calculated as follows:

$$\text{Carbon demand} = \text{NGE} \times \frac{R}{(1-\text{NGE})}$$

The formula for R is given by Eq. (1). Assimilation of bacterial and DOM carbon by the nematodes was calculated as nematode ^{13}C uptake divided by the fractional abundance of ^{13}C in the bacteria and DOM, respectively.

2.3.5. Potential fate of DOM carbon

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

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

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

2.4. Data analysis

All statistical analyses were conducted in Primer v6 (Clarke and Gorley, 2006) with the PERMANOVA+ add-on package (Anderson et al., 2008). Because of the dependency between sediment depth layers within cores, we could not conduct statistical tests to assess differences in environmental conditions or benthic community characteristics between sediment layers (0–1, 1–2, 2–3, 3–4 cm) at seamount site GB1200. For this site, sediment depth-specific patterns were discussed based on graphs and data presented in tables.

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

Benthic community structure was evaluated in terms of bacterial and nematode biomass, as well as nematode genus and feeding type relative biomass composition. Biomass data were obtained from field and experimental samples. Differences between sites in biomass for 0–2 cm sediment depth were investigated using PERMANOVA. Nematode genus and trophic composition were compared between sites (0–2 cm layer) by means of PERMANOVA. For both generic and trophic composition, we used Bray–Curtis dissimilarities based on untransformed, relative biomass data. Where genus or trophic composition differed significantly between groups, a SIMPER analysis identified which genera or feeding types contributed most to the observed dissimilarity and which were typical of the different groups. A non-metric multi-dimensional scaling (nMDS) plot was constructed to visualize differences in genus composition between sites. The PERMDISP routine using distances among centroids was employed to calculate the average dispersion in genus composition within sites (Anderson et al., 2008). For some pairwise PERMANOVA tests, we interpreted the Monte Carlo P-value (P_{MC}) instead of the permutation P-value (P_{PERM}) because the number of possible permutations was < 100.

The results of the isotope enrichment experiments were not evaluated with statistical tests, because of the limited replication. When replicate samples were available, values were reported as mean $\pm$ standard error.

3. Results

3.1. Environmental conditions

The environmental characteristics of all three sites are summarised per sediment layer (0–2 cm for all sites; 0–1, 1–2, 2–3, and 3–4 cm for seamount site GB1200) in Table 2. At seamount site GB1200, there was no clear trend in environmental conditions with sediment depth.

There was a clear distinction in environmental conditions within the 0–2 cm layer between the seamount site and the deeper slope sites (i.e. GB1900 and WMed1900; Fig. 2, Tables 2 and 3). The eigenvector values indicated that all environmental variables contributed more or less equally to axis PC1 (explaining 88.1% of the variation), which marked the separation between the seamount and the slope sites. The most important contributors to the first PCA axis were median grain size (MGS) (−0.48) and the sediment sorting coefficient (SC) (−0.47). The second PCA axis explained 9.0% of the environmental variation and was mostly governed by total organic matter content (TOM) (−0.81).

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

3.2. Characterisation of the benthic community

3.2.1. Nematode and bacterial standing stock

At the seamount site, nematode biomass declined with increasing sediment depth, whereas bacteria displayed subsurface

Table 2
Mean (standard error) values of environmental variables and standing stock of sediment TOC (Sed), bacteria (Bact) and nematodes (Nema) per sediment layer at all sites. Also indicated is the average (standard error) ratio of bacterial to nematode biomass. TN: total nitrogen, TOC: total organic carbon, C:N: molar carbon: nitrogen ratio, TOM: total organic matter, MGS: median grain size, sand: sand content, SC: sediment sorting coefficient. Environmental variables: $n = 3$, except for TOC and C:N at WMed1900, where $n = 1$. Stock concentrations were calculated from experimental cores, with $n = 4$ (GB1200), 7 (GB1900) or 6 (WMed1900)—not determined.

		TN	TOC	C:N	TOM	MGS	Sand	SC	Sed	Biomass (mg C m^{-2})		Biomass
		(%)	(%)	(–)	(%)	(μm)	(%)	(–)	(mg C m^{-2})	Bact	Nema	Bact/Nema
GB1200	0–1 Cm	0.0186 (0.0020)	0.13 (0.02)	8.0 (0.3)	2.13 (0.14)	262.2 (15.1)	91.9 (1.6)	0.601 (0.045)	16397.8 (791.7)	52.4 (2.2)	4.4 (1.3)	14.1 (3.0)
	1–2 Cm	0.0176 (0.0002)	0.11 (0.01)	7.6 (0.5)	2.15 (0.10)	246.1 (7.7)	91.9 (0.9)	0.586 (0.023)	15937.2 (827.9)	55.3 (20.9)	2.8 (0.2)	16.2 (5.8)
	2–3 Cm	0.0202 (0.0015)	0.11 (0.01)	6.4 (0.5)	2.10 (0.05)	262.7 (8.7)	91.4 (0.4)	0.590 (0.005)	15192.9 (671.6)	32.9 (9.6)	1.7 (0.2)	21.5 (7.3)
	3–4 Cm	0.0164 (0.0014)	0.09 (0.01)	6.5 (0.3)	1.96 (0.03)	231.9 (4.2)	91.3 (0.3)	0.616 (0.009)	14303.7 (527.8)	30.0(2.5)	0.8 (0.2)	41.8 (8.9)
	0–2 Cm	0.0181(0.0011)	0.12 (0.02)	7.8 (0.4)	2.14 (0.06)	254.2 (10.0)	91.9 (1.1)	0.593 (0.031)	30914.3 (927.2)	100.7(22.6)	7.2 (1.4)	13.8 (0.9)
GB1900	0–2 Cm	0.0649 (0.0058)	0.51 (0.04)	9.2 (1.1)	7.06 (1.07)	16.4 (1.0)	37.6 (7.6)	1.953 (0.097)	-	229.4 (27.2)	9.7 (1.2)	27.1 (3.8)
WMed1900	0–2 Cm	0.0756 (0.0045)	0.80	11.2	4.59 (0.04)	11.5 (1.0)	19.1 (0.9)	1.651 (0.019)	-	516.8 (72.1)	4.1 (1.2)	158.6 (31.4)

biomass peaks at day 0, 1 and 7 (Fig. 3). In all sediment layers, bacterial biomass exceeded nematode biomass by more than a factor of 10 (Table 2).

Bacterial standing stock in the top 2 cm of the sediment differed significantly amongst sites with WMed1900 > GB1900 > GB1200 (Table 3). Nematode biomass also varied between sites owing to the significantly higher standing stock at GB1900 compared to WMed1900 (Tables 2 and 3). Moreover, the ratio of bacterial to nematode biomass differed significantly amongst sites with WMed1900 > GB1900 > GB1200 (Tables 2 and 3).

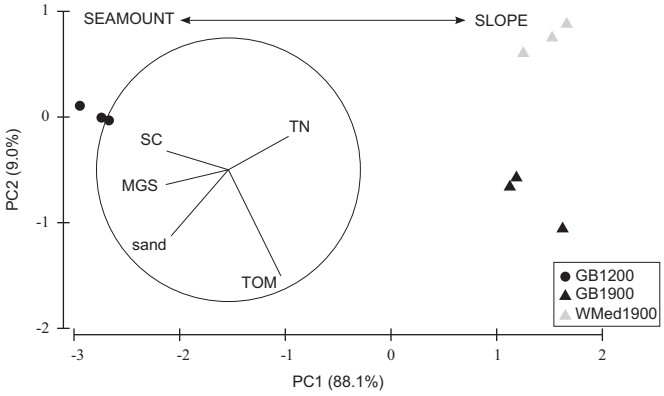

Fig. 2. Principal components analysis (PCA) of normalized environmental variables. Median grain size (MGS) and sand were $\log_e$-transformed to reduce right-skewness, whilst the inverse of the sediment sorting coefficient (SC) was taken to minimize left-skewness. TOM: total organic matter, TN: total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

3.2.2. Nematode community composition

The non-metric multidimensional scaling (nMDS) analysis revealed no substantial differences in nematode genus or trophic composition, based on relative biomass data, between sediment depth layers at seamount site GB1200 (Fig. 4; Table 4). Potential bacterivorous nematodes dominated total nematode biomass (99.4 ± 0.6, 96.1 ± 2.1, 66.3 ± 11.1 and 95.7 ± 2.8% in the 0–1, 1–2, 2–3 and 3–4 cm layers) throughout the sediment column.

There was a clear distinction in genus composition within the 0–2 cm layer between the seamount site (GB1200) and the two slope sites (GB1900 and WMed1900) (Tables 2 and 5; Fig. 5). Fig. 5 indicates that the non-significant difference between the two slope sites is caused by the high within-site variability in genus composition at WMed1900 (average multivariate dispersion is 38.6 and 21.8 for WMed1900 and GB1900, respectively). Several genera typical of the seamount site (e.g. *Trefusia*, *Desmodora*, *Bolbolaimus*, *Microlaimus/Aponema*, *Leptolaimus*) contributed relatively little to nematode biomass at the slope sites (Table 6). In

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

	Main test			**Pairwise tests**		
	df	Pseudo-F	P_{PERM}	GB1200 vs. GB1900 *P*	GB1200 vs. WMed1900 *P*	GB1900 vs. WMed190 *P*
TN	2	50.9	**≤0.01**	**≤0.01**	**≤0.001**	0.22
TOC	2	92.3	**≤0.001**	**≤0.001**	**≤0.001**	0.06
C:N	2	2.4	0.21	–	–	–
TOM	2	46.6	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.05**
MGS	2	562.6	**≤0.01**	**≤0.001**	**≤0.001**	**≤0.05**
Sand	2	72.7	**≤0.01**	**≤0.01**	**≤0.001**	0.07
SC	2	142.4	**≤0.01**	**≤0.001**	**≤0.001**	≤0.05
Bacterial biomass	2	16.6	**≤0.001**	**≤0.01**	**≤0.01**	**≤0.01**
Nematode biomass	2	5.4	**≤0.05**	0.23	0.17	**≤0.01**
Bact/nema biomass	2	15.2	**≤0.001**	**≤ 0.05**	**≤0.01**	**≤0.01**
Genus composition	2	4.5	**≤0.01**	**≤0.01**	**≤0.05**	0.08
Trophic composition	2	10.1	**≤0.01**	0.12	**≤0.05**	**≤0.01**

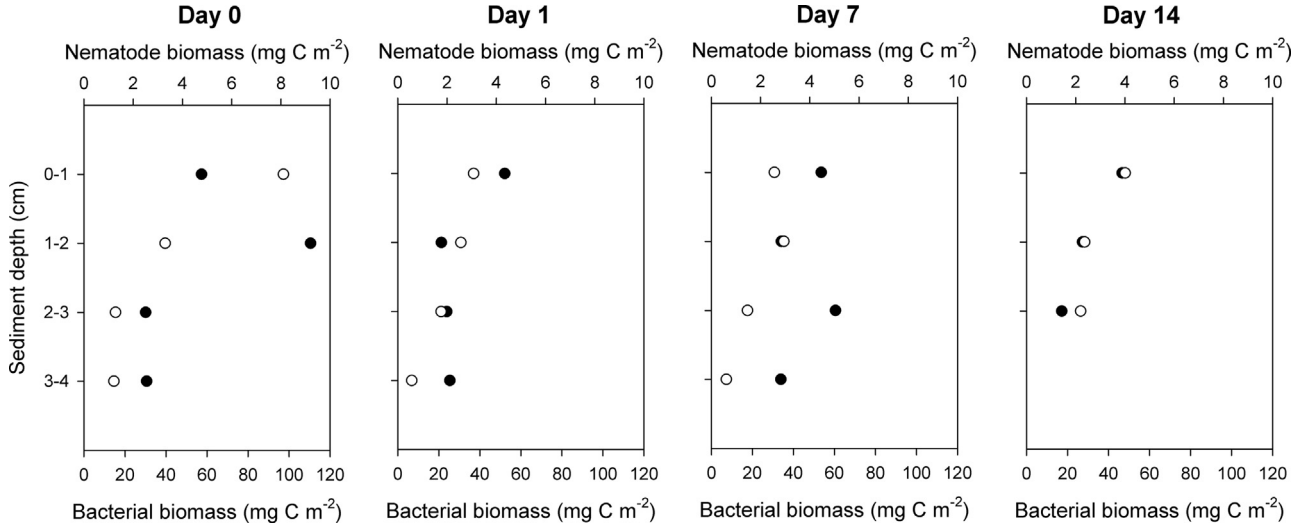

Fig. 3. Nematode (white symbols) and bacterial (black symbols) biomass as a function of sediment depth at seamount site GB1200 per incubation time-step.

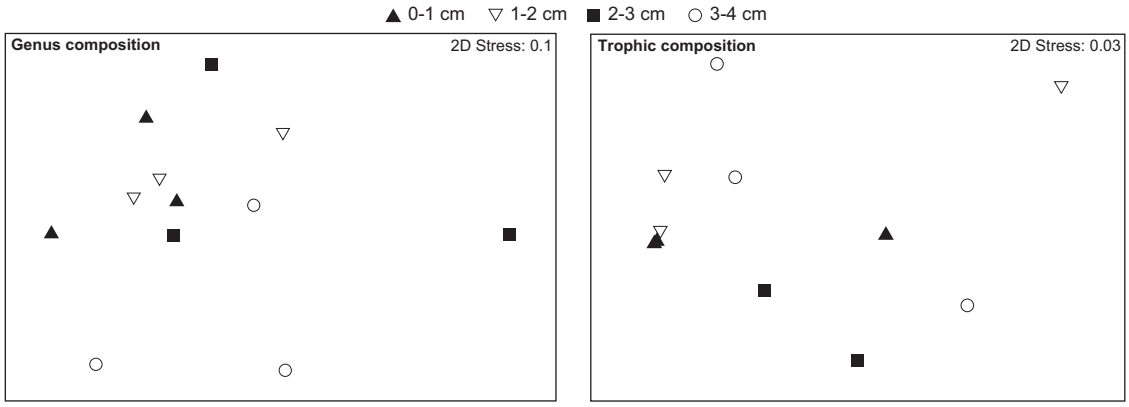

Fig. 4. Non-metric multidimensional scaling (nMDS) plots of nematode genus (left) and trophic (right) composition (based on relative biomass data) per sediment layer at seamount site GB1200.

Table 4
Dominant genera (≥3%) per sediment layer at seamount site GB1200 in terms of average (Standard Error) relative abundance and biomass, with an indication of feeding type (FT) based on buccal morphology according to the scheme by Wieser (1953) (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate feeders, (2B) predators/scavengers.

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

contrast, most genera typifying GB1900 and WMed1900 were also important at one or more of the other sites. Differences in community structure were most pronounced between GB1200 and WMed1900 (average dissimilarity: 76.0%; Table 6). The genera mainly responsible for this disparity were *Sabatieria* (8.1%) and *Bolbolaimus* (7.8%), which dominated nematode biomass at WMed1900 and GB1200, respectively. GB1900 diverged from GB1200 (average dissimilarity: 71.4%) because of the predominance of *Daptonema* and *Acantholaimus*, and the much lower biomass, as well as abundance, of *Desmodora* and *Bolbolaimus* at site GB1900.

The nematode assemblages at the two northeast Atlantic sites were generically distinct, but displayed a similar trophic structure (Table 3, Fig. 4). The feeding type composition at WMed1900 differed from that at the northeast Atlantic sites (Table 3, Fig. 6). The SIMPER analysis indicated that the divergence between WMed1900 and the northeast Atlantic sites was primarily driven by the higher relative biomass of predators/scavengers (2B) at the former site (contribution to dissimilarity, WMed1900–GB1200: 2B: 44.1%, WMed1900–GB1900: 48.8%). Potential bacterivores prevailed at both northeast Atlantic sites (GB1200: $97.5 \pm 12.5\%$, GB1900: $91.9 \pm 0.10\%$). At the Mediterranean slope site, predatory/scavenging nematodes were dominant.

3.3. Isotope enrichment experiments

3.3.1. Isotope dynamics at the seamount site

The temporal dynamics in $\Delta\delta^{13}C$ of nematodes, bacteria and sediment TOC is illustrated in Fig. 7. One day after glucose injection, both nematodes and bacteria had acquired some of the ^{13}C label. At day 1, nematodes were depleted in ^{13}C relative to the sediment, but at days 7 and 14, nematodes were more enriched than sediment TOC in all sediment depth layers ($\Delta\delta^{13}C_{nema}$–$\Delta\delta^{13}C_{sed}$ ranged between 1.5 and 256.0%), indicative of selective feeding.

Table 5
Dominant genera (≥3%) per site in terms of average (standard error) relative abundance and biomass, with an indication of feeding type (FT) according to the scheme of Wieser (1953) (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate feeders, (2B) predators/scavengers.

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

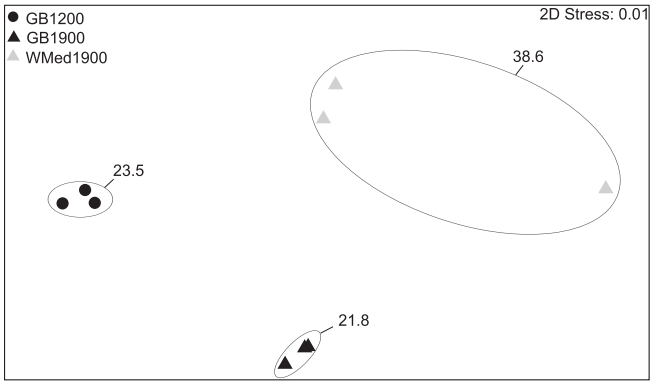

Fig. 5. Non-metric multidimensional scaling (nMDS) plot of relative nematode genus biomass data for 0–2 cm sediment depth at all sampling sites. The ellipses enclose stations sampled at the same site. The numbers indicate the average within-site (or between-station) variability in genus composition, calculated via the PERMDISP routine in PRIMER.

At day 7 (0–1, 1–2, 3–4 cm), in the 3–4 cm layer at day 1, and in the 0–1 cm layer at day 14, nematode $\Delta\delta^{13}C$ surpassed bacterial $\Delta\delta^{13}C$ values (Fig. 7). Consequently, the fraction of nematode carbon derived from bacteria within these sediment layers, as inferred from the $\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$ ratios and under the assumption that only bacteria consumed DOM directly, would exceed 100%. This suggests nematodes were not feeding exclusively on bacterial carbon.

3.3.2. Glucose vs diatom DOM assimilation

At the slope sites, bacteria showed no pronounced preference for diatom DOM or glucose, since the amount assimilated was of the same order of magnitude for both substrates (Fig. 8). At WMed1900, nematodes took up comparable amounts of carbon in both treatments (Fig. 8). In contrast, at GB1900, nematode carbon uptake was almost 15 times higher in the glucose than in the diatom DOM cores (Fig. 8). Nematodes assimilated only 1.8 μg of diatom DOM carbon per m² in one core, while there was no uptake in the other core. Hence, under a DOM feeding strategy, nematodes at site GB1900 preferred glucose over diatom DOM. Alternatively, under the bacterivorous strategy, nematodes discriminated between bacteria feeding on diatom DOM and those feeding on glucose.

3.3.3. The importance of DOM and bacteria in the nematode diet

Assuming a DOM feeding strategy, the seamount nematode assemblage would have derived a very limited part of their carbon from glucose (max. 0.33%, Table 7). The share of bacterial carbon in the nematode diet ($\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$) surpassed that of glucose, and often exceeded 100% (see Table 7). The amount of glucose assimilated was never sufficient to balance respiration losses; it accounted for a maximum of 1.3% (day 1, 3–4 cm) of the necessary amount. Compared to the DOM-feeding strategy, the bacterivorous strategy resulted in a greater fulfilment of carbon needs (Table 7). The two methods we used to infer the share of DOM and bacteria in the nematode diet both showed highly variable values over the 0–4 cm sediment column at the seamount site, revealing no consistent trend with sediment depth.

GB1200 was the only site where after 7 days nematodes were more enriched in ^{13}C than bacteria in the 0–2 cm layer (Fig. 7; data not shown for the slope sites). At all sites, nematodes assimilated much more bacterial carbon, under a bacterivorous strategy, than DOM carbon, under the DOM feeding strategy (Fig. 9). Consequently, the fraction of carbon derived from DOM (always < 1%, Table 7) was much less than that derived from bacteria (ranging between 0.8 and 141.2%; Table 7). In the 0–2 cm layer at site GB1200, the potential dietary contribution of bacteria was 700 times higher than that of glucose and amounted to 141.2%. At the slope sites, nematodes would have derived ~35% more carbon from bacteria (Table 7) than from DOM.

At all three sites, neither bacterial nor DOM assimilation fulfilled nematode carbon requirements over a time span of 7 days (< 100%; Table 7). The seamount nematode assemblage incorporated more carbon than the nematodes at the slope sites, which displayed similar carbon assimilation values (Fig. 10). Hence, nematodes from the seamount site were most successful in meeting their carbon demands (feeding on DOM: 0.5%, feeding on bacteria: 36.9%; Table 7). In comparison, the DOM feeding and bacterivorous strategies at the two slope sites contributed at most 0.1% and 0.1–1.7%, respectively, to theoretical nematode carbon

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

GB1200	
Average similarity: 59.3%	
Desmodora	12.5%
Trefusia	11.6%
Leptolaimus	10.7%
Halalaimus	9.0%
Bolbolaimus	8.2%
Microlaimus/Aponema	6.2%
Acantholaimus	5.7%
Richtersia	5.1%
Metadesmolaimus	3.9%

GB1200–GB1900	
Average dissimilarity: 71.4%	
Daptonema	9.1%
Bolbolaimus	8.3%
Acantholaimus	7.2%
Trefusia	6.7%
Desmodora	6.5%
Leptolaimus	4.0%
Richtersia	3.9%

GB1900	
Average similarity: 62.2%	
Acantholaimus	22.0%
Daptonema	20.3%
Halalaimus	10.9%
Desmoscolex	6.0%
Sabatieria	4.8%
Diplopeltula	4.5%
Halichoanolaimus	3.2%

GB1200–WMed1900	
Average dissimilarity: 76.0%	
Sabatieria	8.1%
Bolbolaimus	7.8%
Desmodora	7.5%
Epsilonema	7.3%
Metasphaerolaimus	6.0%
Trefusia	5.8%
Leptolaimus	4.2%
Richtersia	3.0%

GB1900–WMed1900	
Average dissimilarity: 68.4%	
Trissonchulus	11.3%
Daptonema	9.3%
Acantholaimus	6.9%
Sabatieria	6.9%
Richtersia	4.5%
Halalaimus	4.2%

WMed1900	
Average similarity: 33.2%	
Sabatieria	14.9%
Acantholaimus	14.5%
Cervonema	9.7%
Halalaimus	9.1%
Metasphaerolaimus	7.2%
Sphaerolaimus	5.1%
Halichoanolaimus	4.8%
Neochromadora	4.6%
Spirobolbolaimus	3.0%

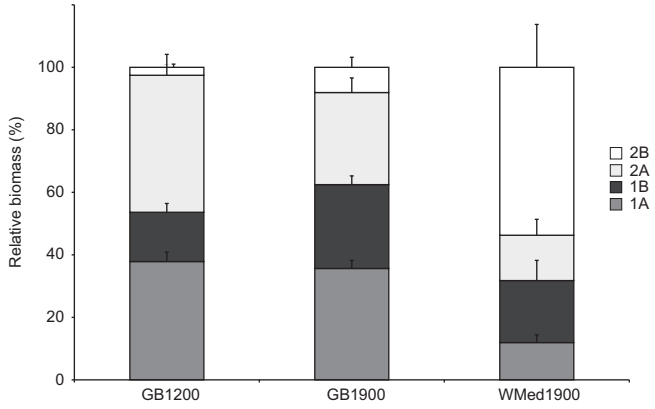

Fig. 6. Nematode trophic composition (inferred from the relative biomass of feeding types) at 0–2 cm sediment depth per site. (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate feeders, (2B) predators/scavengers. Error bars denote Standard Error.

requirements. Bacterivory always resulted in a greater fulfilment of carbon demands than the DOM feeding strategy (Table 7).

3.3.4. Potential fate of DOM carbon

To quantify maximal potential flows of added DOM carbon, we presumed nematodes fed exclusively on DOM (i.e. the DOM feeding strategy), although the potential role of nematodes in the flux of DOM carbon was very limited. Since the nematode community at GB1900 assimilated more carbon in glucose amended than in diatom DOM amended cores, we plotted nematode carbon uptake at GB1900 for each treatment separately (Fig. 8). Bacteria assimilated more DOM-derived carbon compared to nematodes at all three sites (a factor of 10–1000), and were responsible for at least 92% of total uptake (Fig. 10). However, per unit biomass, nematodes incorporated 1.5 times more glucose carbon than the bacteria at the seamount site (Fig. 10). At the slope sites, biomass-specific assimilation was higher for bacteria than for nematodes. The contribution of nematodes to total DOM assimilation at site GB1900 was elevated in the glucose cores ($0.67 \pm 1.53 \times 10^{-5}$%) compared to the diatom DOM cores ($0.02 \pm 3.26 \times 10^{-5}$%). The share of nematodes in total DOM assimilation was ca. 10 and 50 times higher at seamount site GB1200 (8.06%) relative to GB1900 (glucose: $0.67 \pm 1.53 \times 10^{-5}$%, diatom DOM: $0.02 \pm 3.26 \times 10^{-5}$%) and WMed1900 ($0.14 \pm 0.06$%), respectively.

Most of the DOM carbon added was not processed by the bacteria and nematodes (Fig. 11). The fraction of DOM processed (i.e. assimilated, defecated or respired) by the bacteria and the nematodes was highest at WMed1900 (41.9 ± 14.0%), lower at GB1900 (13.4 ± 3.1%) and lowest at GB1200 (4.8%). Assimilation was a minor pathway of DOM carbon processing and accounted at most for 0.26–2.10% of the added DOM. The greatest fraction of DOM carbon was assimilated at WMed1900 (2.1 ± 0.7%), followed by GB1900 (0.7 ± 0.2%), and then GB1200 (0.3%). Respiration was the major fate of processed carbon at all sites (Fig. 11). ^{13}C-enrichment of the sediment sampled at GB1200 showed that 20.7% of the unprocessed carbon was present in the sediment pool.

4. Discussion

4.1. Benthic community composition in relation to environmental conditions

The environmental conditions at the seamount site contrasted strongly with those at the slope sites. Compared to the slope, seamount sediments were coarser, sandier, and better sorted,

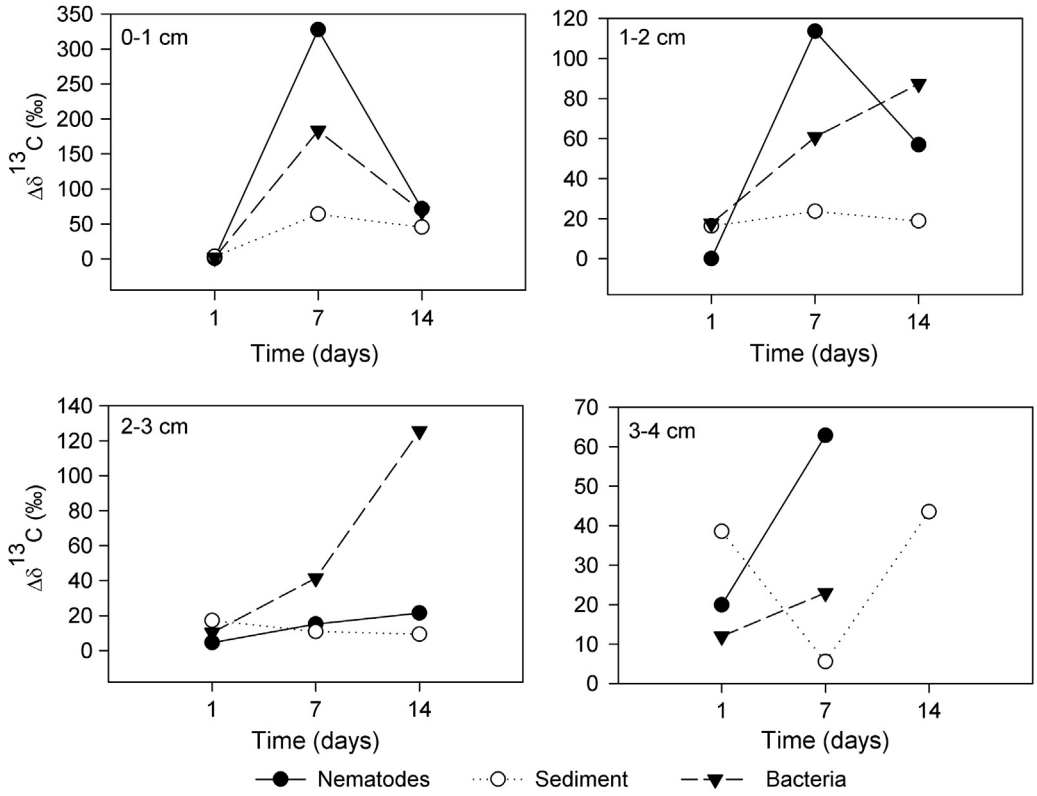

Fig. 7. Temporal dynamics of the $\Delta\delta^{13}C$ values of nematodes, bacteria and sediment TOC per sediment depth layer at seamount site GB1200.

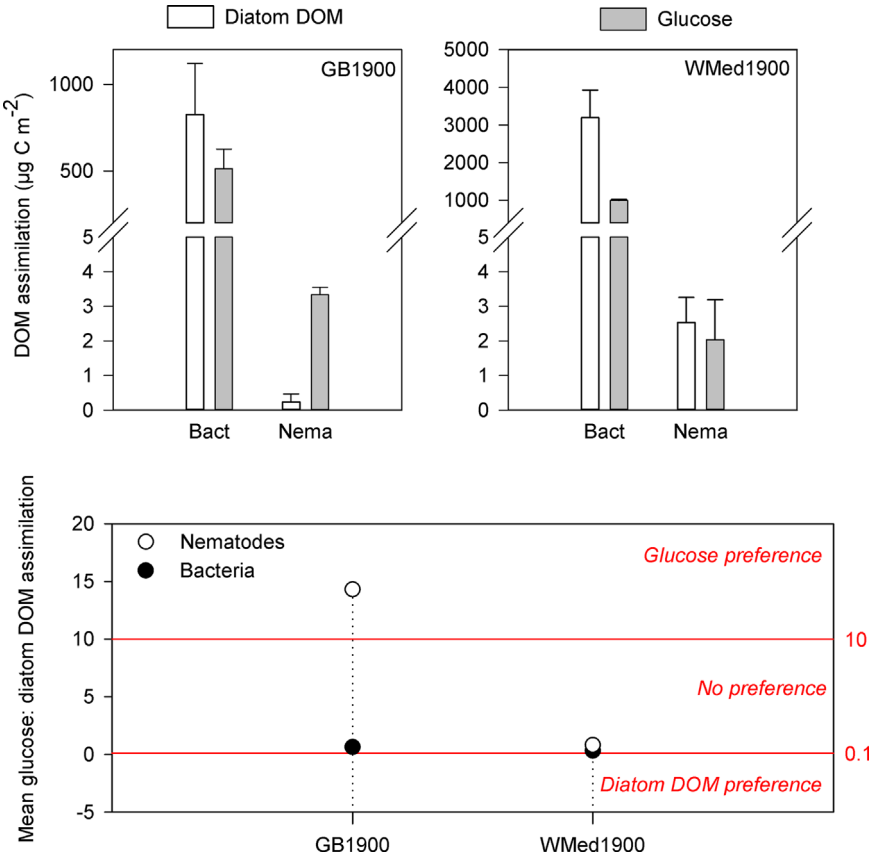

Fig. 8. Diatom DOM vs. glucose assimilation by bacteria (bact) and nematodes (nema) at slope sites GB1900 and WMed1900 after 7 days in the 0–2 cm layer. Top: average assimilation of glucose and diatom DOM carbon. Error bars denote Standard Error. Bottom: Dietary preference of glucose vs. diatom DOM. The lower red line denotes the situation where glucose assimilation is 10 times less than diatom DOM assimilation, while the upper red line indicates a 10-fold higher assimilation of glucose relative to diatom DOM. The zone between the red lines represents the case of no apparent preferential assimilation. (For interpretation of the references to colour in this figure legend, the reader is referred the to web version of this article.)

Table 7
Potential contribution (%) of DOM (glucose or diatom DOM) and bacteria to the nematode diet at each site. For site GB1900, the results for the glucose and the diatom DOM cores are presented separately because of the pronounced difference in carbon assimilation between these two treatments. Assimilation/carbon demand represents the ratio of observed assimilation ("assimilation") vs. assimilation required to fulfil theoretical carbon demands ("carbon demand"). Values are averages (standard error). At GB1200, only one core was sampled per time-step.

		DOM				**Bacteria**			
		$\Delta\delta^{13}C_{nema}/\delta^{13}C_{DOM}$		Assimilation/carbon demand		$\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$		Assimilation/carbon demand	
GB1200									
day 1	0-1 cm	0.0008		0.05		47.8		4.7	
	1-2 cm	0.0000		0.00		0.0		0.0	
	2-3 cm	0.0046		0.99		44.8		89.0	
	3-4 cm	0.0199		1.34		166.9		119.7	
day 7	0-1 cm	0.3276		0.57		178.8		43.7	
	1-2 cm	0.1136		0.36		186.8		30.6	
	2-3 cm	0.0152		0.04		36.6		3.5	
	3-4 cm	0.0628		0.14		273.3		12.8	
day 14	0-1 cm	0.0714		0.10		105.2		8.3	
	1-2 cm	0.0569		0.07		65.1		6.0	
	2-3 cm	0.0215		0.04		17.1		3.4	
day 7	0-2 cm	0.2		0.5		141.2		36.9	
GB1900									
day 7	0-2 cm	Glucose:	0.55 (0.02)	Glucose:	0.126 (0.008)	Glucose:	19.46 (0.06)	Glucose	1.691 (0.110)
		Diatom DOM:	0.03 (0.03)	Diatom DOM:	0.009 (0.009)	Diatom DOM:	0.75 (0.75)	Diatom DOM	0.086 (0.086)
WMed1900									
day 7	0-2 cm	0.8 (0.3)	0.114 (0.029)	29.8 (16.5)	1.290 (0.349)				

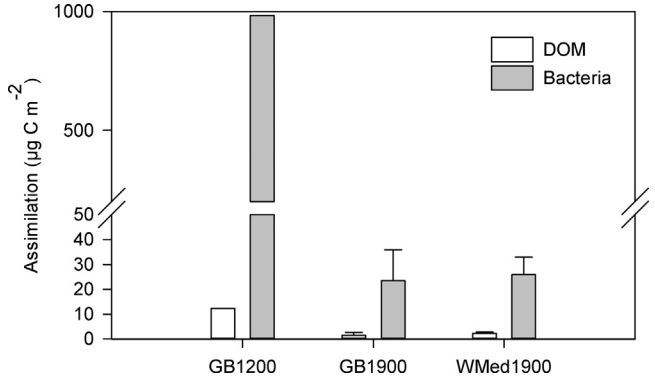

Fig. 9. Nematode carbon assimilation under a DOM feeding (white bars) and a bacterivorous (grey bars) strategy at 0–2 cm sediment depth. DOM assimilation represents average assimilation of diatom DOM and glucose at slope sites GB1900 and WMed1900. Error bars denote Standard Error.

indicating a high-energy environment (Duineveld et al., 2004; van Weering et al., 2002). The seamount site harboured harboured a distinctive nematode genus composition compared to the slope sites, which may be related to the hydrodynamic regime and/or water depth. In a review of nematode community data from different deep-sea macrohabitats, a clear difference was observed between slope and seamount sites, the latter being dominated by genera like *Desmodora*, *Richtersia* and *Ceramonema* (Vanreusel et al., 2010). These first two genera were also prominent at our seamount site. Vanreusel et al. (2010) suggested that the dominance of these genera, which possess coarsely ornamented cuticles, may be related to the sediment micro-structure. The difference in geomorphology between seamount site GB1200 and the two slope sites (i.e. GB1900 and WMed1900) was not mirrored in a differential nematode trophic structure, since the communities at GB1200 and GB1900 had a similar feeding type composition. In contrast, the seamount site and slope site WMed1900 differed in trophic structure. Hence, the geomorphologic setting is merely one of several potential factors driving the trophic composition of deep-sea nematode communities.

4.2. Feeding strategies of deep-sea nematodes

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

After 7 days, the seamount nematode assemblage was more enriched in ^{13}C than the sediments and the benthic bacteria. The higher ^{13}C enrichment of nematodes relative to the sediment suggests that they feed selectively, contrasting with Guilini et al. (2010). The higher nematode vs bacterial enrichment at the seamount points to the possibility of direct glucose uptake by the nematodes. Glucose uptake has been demonstrated for shallow-water nematodes by Chia and Warwick (1969) and Montagna (1984). However, a bacterivorous strategy at seamount site GB1200 is also plausible if bacterial $\Delta\delta^{13}C$ peaked before day 1, or between day 1 and 7 after glucose injection. Guilini et al. (2010) observed maximal bacterial enrichment 2–4 days after glucose injection. Hence, considering a time lag between the consumption of labeled bacteria and the appearance of the ^{13}C label in nematode tissue, bacterivory is a possible feeding scenario. Nonetheless, this is the first feeding study to show that direct DOM feeding in deep-sea nematodes is possible.

Although isotope dynamics indicated that direct DOM uptake is a plausible nematode feeding strategy at the seamount site, our

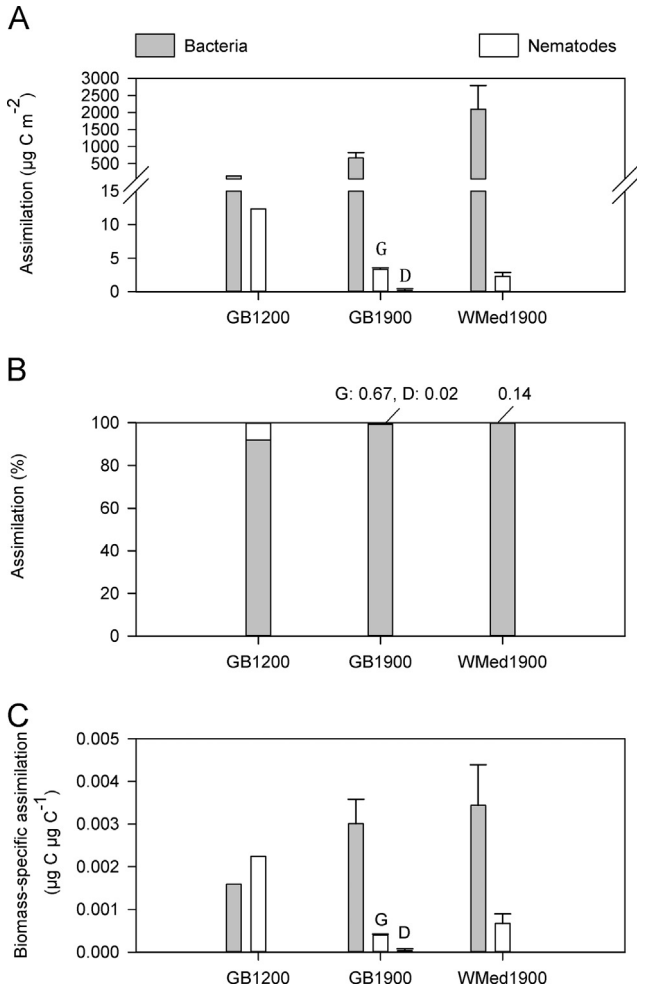

Fig. 10. (A) Total assimilation, (B) assimilation partitioning and (C) biomass-specific assimilation by bacteria (grey bars) and nematodes (white bars) per site. For nematodes at site GB1900, carbon assimilation in the glucose (G) and the diatom DOM (D) cores are presented separately. At site WMed1900, the DOM assimilation averaged over both treatments is depicted. At GB1900 and WMed1900, the potential share of nematodes in total DOM carbon assimilation was negligible and therefore the actual values are given above the bars (B). Error bars denote Standard Error.

data suggest that DOM is a potentially relatively unimportant food source for the nematode community. The amount of nematode carbon derived from DOM, and the contribution of DOM assimilation to theoretical nematode carbon demands, was mostly less than 1%. Bacteria constituted a potentially very important carbon source, since they possibly were the source of a substantial part of the nematode carbon (which was occasionally well over 100%) and bacterivory potentially contributed between 0 and 120% to carbon requirements. However, the higher ^{13}C enrichment of nematodes vs bacteria, and the fact that carbon requirements were not fulfilled, indicates that the diet of nematodes at the seamount included, besides bacteria, other ^{13}C-labeled items like DOM. As at the seamount site, the potential importance of DOM in the nematode diet at slope sites GB1900 and WMed1900 was negligible. Employing a bacterivorous strategy, nematodes derived a maximum of 20% in the glucose amended cores at GB1900, and almost 30% at WMed1900 of their carbon from bacteria (as inferred from $\Delta\delta^{13}C_{nema}/\Delta\delta^{13}C_{bact}$). This together with the fact that bacterial feeding contributed a maximum of 1.7% to carbon requirements (as inferred from biomass-specific respiration rates) suggests that bacteria were probably not the primary food source for nematodes at the slope sites.

The differential assimilation rates between sites point to an influence of the environment and/or community composition on nematode feeding behaviour. Compared to the slope sediments, the sandy seamount sediments had a low amount of organic matter and nitrogen, and low bacterial biomass (see Table 2). Thus, the more rapid response of the seamount vs the slope nematodes may reflect an adaptation to the food-poor environment. Coastal nematodes showed higher uptake of ^{13}C-enriched cyanobacteria (Urban-Malinga and Moens, 2006) and algae (Franco et al., 2008) in coarser than in finer sediments. It should be noted that the difference in timing, and thus in the amount of in situ organic matter deposition (see Fig. 2B in Pape et al., 2013), between the seamount (spring) and the slope experiments (autumn) may have contributed to the discrepancy in carbon uptake rates. The higher assimilation rates at the seamount may be related to the enhanced metabolic activity during sedimentation in spring (Pfannkuche, 1993).

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

Guilini et al. (2010) and Gontikaki et al. (2011b) reported a limited contribution of bacterivory to nematode carbon requirements amounting to 0.1–5.1% at the Arctic Hausgarten Site and 0.74% in the sub-Arctic Faroe-Shetland channel. In comparison, bacterivory at the seamount site, where we used the same experimental methodology as Guilini et al. (2010), resulted in a greater potential fulfilment of carbon demands (ranging between 0 and 119.7% over 0–4 cm sediment depth). At the slope sites, the contribution of bacterial assimilation to nematode metabolic requirements (0.1–1.7%) was as low as that reported by Guilini et al. (2010) and Gontikaki et al. (2011b). Because it is difficult to measure nematode respiration in situ, the present study and that of Guilini et al. (2010) and Gontikaki et al. (2011b), inferred nematode carbon demands from biomass-dependent respiration following de Bovée et al. (1990). This formula was generated with data from several metabolic studies conducted in freshwater and coastal environments (see references in de Bovée and Labat (1993). Hence, the validity of the formula in the deep sea needs

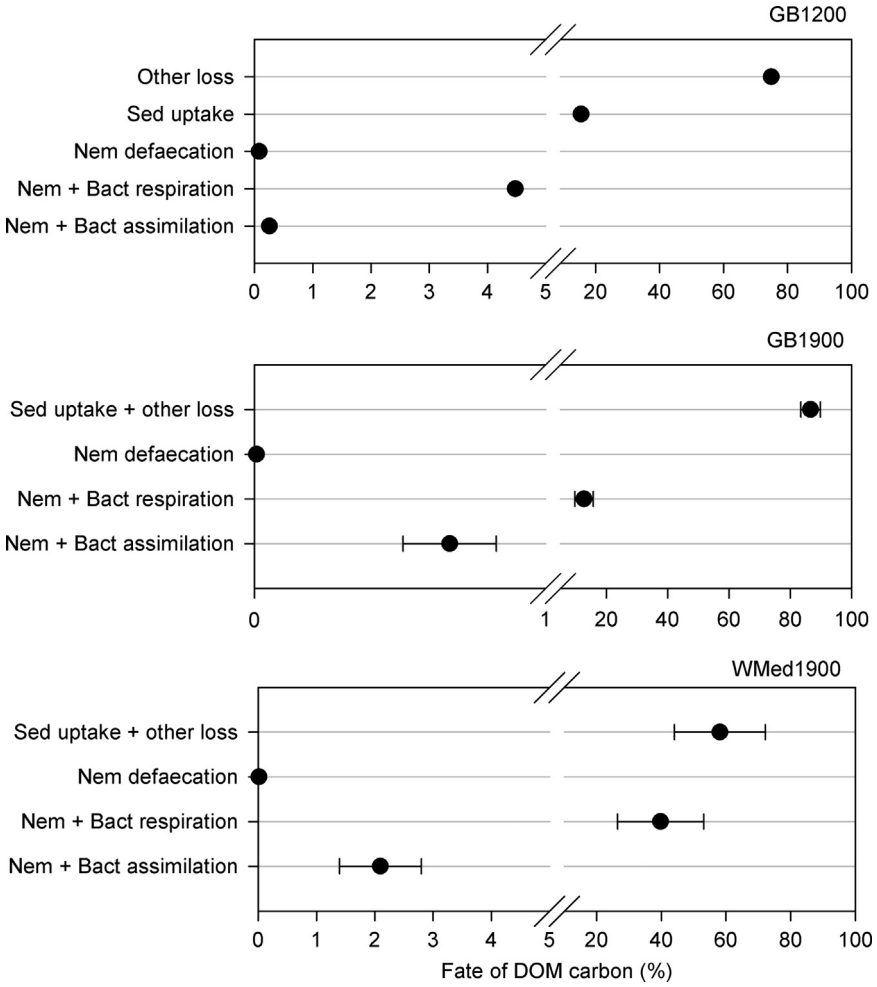

Fig. 11. Fate of added DOM carbon at 0–2 cm sediment depth at the seamount (GB1200) and slope sites (GB1900 and WMed1900). Nem: nematode, Bact: bacterial, Sed: sediment. Error bars denote Standard Error. At the seamount site, only one station was sampled.

to be evaluated on the basis of in situ respiration measurements. Braeckman et al. (2013) showed that the respiration rate of an intertidal predatory nematode species decreased with lower oxygen availability. Assuming a similar oxygen-concentration dependence of respiration rates in the deep sea, these authors concluded that nematode respiration at the deep Arctic Hausgarten site, calculated according to de Bovée et al. (1990), was overestimated by a factor of two when the formula was not corrected for ambient, lower oxygen concentration. If nematode respiration is truly overestimated, nematode carbon assimilation observed in the experiments would have resulted in a greater fulfilment of carbon demands. Furthermore, we calculated minimal nematode carbon demands based on a minimal net growth efficiency (NGE) of 0.6, observed in coastal nematodes (see references in van Oevelen et al., 2006c). Bacteria in food-limited environments display low bacterial growth efficiency (BGE; del Giorgio and Cole, 1998), and possibly NGE is related to food availability as well. Nevertheless, nematode carbon demands may be realistic and the potential food sources that were labeled here, i.e. bacteria and labile DOM, may not be utilized to a large extent by nematodes. Modelling benthic carbon flows, Gontikaki et al. (2011c) and van Oevelen et al. (2012) concluded that semi-labile detritus was the main food source for deep-sea nematodes. To date, however, no experiments have been conducted to confirm this finding.

In environments with a considerable share of refractory organic matter, it was predicted by Jumars et al. (1990) for macro- and megafauna, and later demonstrated by Hall and Meyer, 1998 for stream macro-invertebrates, that deposit feeders rely greatly on bacteria to meet their carbon and energy demands. Compared to macro- and megafauna, deposit-feeding nematodes possess smaller buccal cavities, implying a more selective feeding behaviour. It follows that, in refractory settings, bacterivory might be more common in nematodes than in larger deposit feeders. The Algero-Provençal basin (WMed1900) experiences reduced primary productivity (153 g C m^{-2} yr^{-1}; Bosc et al., 2004) compared to the north-western Iberian margin (GB1900; 220 g C m^{-2} yr^{-1}; Joint et al., 2002). Thus, we expected a higher reliance of nematodes on bacterial carbon at WMed1900 relative to GB1900, but also at greater vs shallower sediment depths at the seamount. Our results indicated no difference in the potential trophic significance of bacteria to nematodes between the Mediterranean and northeast Atlantic slope sites, and between sediment depth layers at the seamount site. A similar lack of sediment depth-specific feeding behaviour was observed for coastal nematodes (van Oevelen et al., 2006b). The absence of a sediment-depth related trend was governed by the high variability in nematode $\delta^{13}C$. Similarly, Ingels et al. (2010) noted high variability in nematode ^{13}C enrichment between replicate samples. The hypothesis of an elevated reliance on bacterial carbon in more refractory environments (Hall and Meyer, 1998; Jumars et al., 1990) was thus not confirmed here.

The scheme of Wieser (1953) was not always consistent with observed nematode carbon assimilation rates. Based on buccal morphology, potentially bacterivorous nematodes dominated both

northeast Atlantic sites (> 90% of total nematode biomass). However, the experimental results implied that bacteria were only potentially important for the seamount assemblage. Despite the divergent feeding type composition between GB1900 and WMed1900, carbon assimilation was comparable between these two sites. The former site had a higher proportion of non-selective deposit feeders, whereas the latter harboured more predators/scavengers. Hence, assuming that predators/scavengers do not feed on bacteria and selective deposit feeders primarily do, buccal morphology actually implies a lower bacterial dependence at the supposedly more refractory environment of the Mediterranean slope site. The lack of variation in carbon uptake with sediment depth at the seamount was in agreement with the feeding type classification, since genus (and hence feeding type) composition was comparable between sediment layers.

4.3. Fate of DOM carbon

In a feeding experiment at Sagami Bay (1453 m), at least half of the added glucose carbon was respired after 9 days (Nomaki et al., 2011). In the present study, we approximated respiration rates on the basis of biomass and growth efficiencies, and found a similar, dominant role for respiration in DOM carbon processing and only a little assimilation over a time span of 7 days at all sites. The amount assimilated by the bacteria and the nematodes (max. 2.1% of the added DOM substrate) was in the same range as the values of Guilini et al. (2010) (1.6–5.1%). Since bacterial standing stock largely exceeded nematode standing stock, bacteria dominated DOM carbon uptake. Considering the DOM feeding strategy for the nematodes, bacteria had incorporated much more DOM carbon than nematodes after 1 week, as observed after 10 days for intertidal sediments by van Oevelen et al. (2006a). However, at the seamount site, nematodes assimilated more carbon per unit biomass than bacteria, in contrast with the slope sites and van Oevelen et al. (2006a). This result implies that the secondary production rate of nematodes over a period of 7 days exceeded that of bacteria, which is unprecedented.

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

In several experiments with labile particulate organic matter (POM), a substantial part of the added carbon was not processed by the benthos (Moodley et al., 2002; Woulds et al., 2009, 2007). Labile DOM is expected to be taken up more readily than POM, since bacteria need to produce and excrete enzymes to break down POM before it can be transported through the cell wall. Nevertheless, the bulk of the added DOM was left unprocessed by the bacteria and the nematodes (60–88% on average). A similar observation was made by Nomaki et al. (2011). Sediment ^{13}C labelling at the seamount site showed that about 20% of this "lost" label was still present in the sediment, suggesting that a considerable part of the added glucose was adsorbed to the sediment particles (Henrichs and Sugai. 1993; Keil et al., 1994; van Oevelen et al., 2006a). Given the coarse seamount sediments and the inverse relationship between adsorption and grain size (Mayer, 1994), an even greater proportion of the added DOM would have been protected from mineralisation within the fine-grained slope sediments. However, sediment ^{13}C "uptake" also may have resulted from the DOM uptake by other benthic biota like protozoans (including foraminiferans) and macrofauna. However, after 9 days, Nomaki et al. (2011) detected very little and no glucose assimilation by foraminiferans and copepods, and by meiofauna-sized polychaetes and macrofaunal cumaceans, respectively. Notwithstanding the considerable ^{13}C enrichment of the sediment pool, most DOM carbon was lost from the seamount sediments through diffusion to the upper water column or the deeper sediment layers.

5. Conclusions

This is the first nematode feeding study to demonstrate higher ^{13}C labelling of nematodes compared with bacteria and bulk sediment organic matter. Although the isotope dynamics in the time-series experiment showed that DOM and bacteria were both plausible nematode food sources, the contribution to nematode secondary production and theoretical metabolic requirements revealed that bacteria represented a more important potential food source than DOM at all three sites. The higher carbon assimilation rates displayed by the seamount vs the slope nematode assemblages may reflect an adaptation to the food-poor environment. Our data also showed that the potential trophic significance of bacteria to the nematode diet was related to the environment, though apparently not to the share of refractory organic matter, and the associated community composition. Furthermore, we noted that the carbon assimilation rates observed in the experiments were not always supported by the feeding type composition based on buccal morphology. Sites with a similar trophic structure showed large differences in nematode label assimilation, whilst sites with a dissimilar feeding type composition showed comparable uptake. The majority of added DOM carbon was not processed by the small-sized, benthic biota studied. The major fate of processed DOM carbon was respiration.

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