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

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Abstract

A stable isotope (^{13}C)-labeling experiment was performed to quantify the importance of bacterial carbon as a food source for an Arctic deep-sea nematode community. Bacterial functional groups were isotopically enriched with ^{13}C-glucose, ^{13}C-acetate, ^{13}C-bicarbonate, and ^{13}C-amino acids injected into sediments collected from 1280 m depth at 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. The different substrates clearly labeled different bacterial groups as evidenced by differential labeling of the PLFA patterns. The deep-sea nematode community incorporated a very limited amount of the label, and the isotope turnover model showed that the dynamics of the isotope transfer could not be attributed to bacterivory. The low enrichment of nematodes suggests a limited passive uptake of injected ^{13}C-labeled substrates. The lack of accumulation suggests that the injected ^{13}C-labeled dissolved organic carbon compounds are not important as 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 unclear.

Most deep-sea benthic life depends on photosynthetically derived particulate organic matter that sinks from the surface waters to fulfill 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 (Graf 1992; Pfannkuche 1993; Soltwedel 1997). Despite the overall control of detritus input on the benthic food web, it has proved to be more difficult to relate changes in the metazoan standing stock to seasonal inputs of phytodetritus (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 timescales as compared with the seasonality of particulate organic carbon (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, with assimilation within 36 h

(Moodley et al. 2002; Witte et al. 2003*a*) to 8 d (Witte et al. 2003*b*). 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. in press (*a*)) or absent (Nomaki et al. 2005; Sweetman and Witte 2008; Ingels et al. in press (*b*)). Occasionally a time delay of around 3 weeks prior to a limited uptake was observed for nematodes (Witte et al. 2003*b*). Since 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 classified as selective deposit feeders or microvores, according to their unarmed, small buccal cavity and ecological characteristics (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 δ^{15}N values of nematodes to be well above δ^{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

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or activity and nematode abundance over a sediment vertical profile and a bathymetric gradient, respectively, in deep-sea sediments (Vanreusel et al. 1995; 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 originate from experiments where freeze-dried bacteria were offered to the benthos and only minute uptake by nematodes was observed (Ingels et al. in press (*a* and *b*)).

Isotope tracer studies in streams (Hall and Meyer 1998; Hamilton et al. 2004) and intertidal sediments (Van Oevelen et al. 2006*a,b*), in which the microbial compartment was isotopically enriched with a dissolved inorganic or organic substrate, have successfully traced bacterial nitrogen or carbon in potential 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. 2006*a*). In these studies, bacteria were represented as one functional group in the food webs, 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 in quantifying 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. Since 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 dynamics of the nematodes were 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?

Methods

Study site and experimental approach—The experiment was conducted on board of the German ice-breaker R/V *Polarstern* during the ARK XXII-1c campaign to the Hausgarten site in July 2007. The Hausgarten site 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), were immediately transferred to a cold room at in situ temperature (-0.8°C), and were kept oxygenated by air bubbling. From three 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. comm.), and the sediment organic carbon content, determined from the experimental control cores, was on average 1.5% or 70 g m^{-2}. After 12 h of acclimatization, the ^{13}C-labeled substrates (acetate, "^{13}C-ACE"; bicarbonate [dissolved inorganic carbon], "^{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 with 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 setup and manipulation of the cores on board. At four time steps (days 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 δ^{13}C of PLFAs, for δ^{13}C of particulate organic carbon (POC),

which includes dissolved organic carbon (DOC), for $\delta^{13}C$ of nematodes, and for the nematode community composition. We gave priority to $\delta^{13}C$ samples, and therefore we 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 analyzed. An elemental analyzer (EA) isotope ratio mass spectrometer (IRMS) was used to measure stable isotope ratios and carbon content of nematodes. Carbon contents were 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 5 cm from the control cores, another maximum of 120 nematodes were handpicked randomly, transferred to anhydrous glycerine, 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), nonselective deposit feeders (1B), epigrowth feeders (2A), and predators or 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 and measured by gas chromatography–IRMS for PLFA isotope values (details in Middelburg et al. 2000; Van Oevelen et al. 2006a). The bacterial isotope signature was determined from the weighted average bacterial PLFA biomarkers (typically saturated and monounsaturated 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 Belmnite (VPDB) as reference standard and expressed in units per thousand (‰), 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 with 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 = ($F_{sample} - F_{background}$) × 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):

$$d\Delta\delta^{13}C_{NEM}/dt = k_c B_{dep}\Delta\delta^{13}C_{BAC} - k_c\Delta\delta^{13}C_{NEM} \quad (1)$$

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 intervals 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 requirement 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, Flexible Modeling Environment (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 is derived from an estimated parameter covariance matrix by linearizing around the optimal parameter values (Soetaert and Petzoldt 2010). Parameter B_{dep} was allowed to range between 0 and 1. 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 (dry wt, μg C ind^{-1}) for deep-water benthic metazoans:

$$R = 0.0174 \text{ dry wt}^{-0.24} \quad (2)$$

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

$$R = 0.0449 \text{ dry wt}^{-0.1456} \exp^{\ln(Q10)/10(T-20)} \quad (3)$$

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

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 is given as an average of replicates $\pm$ SD).

Depth (cm)	Nematode dry weight (μg C)	Biomass-specific respiration (d^{-1})	
		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

of nematodes in a sample cup. The k_c parameter range was chosen based on these results (Table 1) and was set to 0.01–0.03 d^{-1}.

Statistics—Statistical analyses were conducted using the Primer version 6 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. Nonmetric analysis of similarities (ANOSIM), multidimensional scaling (MDS), and principal component analysis (PCA) were used to detect and visualize patterns in the nematode and bacterial communities. The two-way crossed ANOSIM for nonreplicated 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 whether the differences of the nonreplicated 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 ($p > 0.001$) based on the time-step grouping and clarified between the treatments in which the significant differences were found.

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. per 10 cm² 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 ($R \leq 0.325, p \geq 0.012$). 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 ($R \geq 0.558, p = 0.001$). The
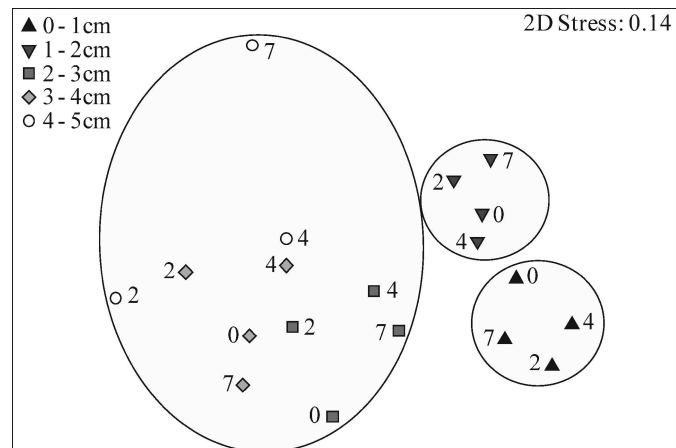

Fig. 1. Two-dimensional MDS plot of the nematode community present in the control cores over 5-cm of depth, with indication of the sampling time step (days 0, 2, 4, and 7).

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). Of the total number of nematodes, 44.3% were 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 dry 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 nonselective 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.

^{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 significant difference in biomass neither between the different treatments and across the time steps, nor between the time steps and across the different treatments ($R \leq 0.091, p \geq 0.26$). 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 downward to 16% in the 4–5-cm sediment horizon. Based on the bacterial biomasses, the nematode abundances, and their biomass-specific respira-

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%			

tion, it was calculated that 0.03–0.22% of the bacterial standing stock would be sufficient to meet nematodes' 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 1 d after injection and down to 5-cm depth. Total bacterial label incorporation was maximal typically at days 2 and 4 after injection and highest in the top 2 cm (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
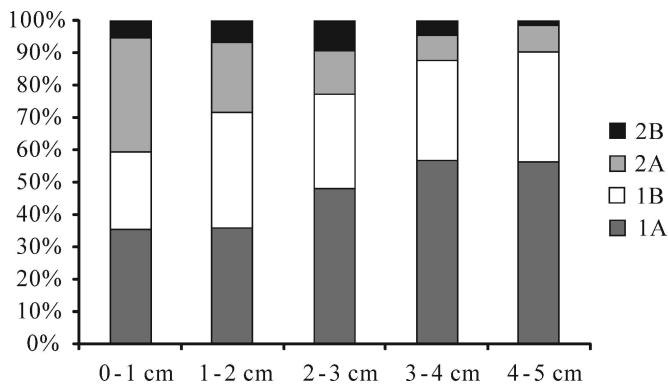

Fig. 2. Relative abundance (%) of the feeding guilds according to Wieser (1953): selective deposit feeders (1A), nonselective deposit feeders (1B), epigrowth feeders (2A), and predators or scavengers (2B).

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 with the other treatments and never exceeded 130 μg ^{13}C m^{-2} (0.07%) and was only observed in the upper two and lower centimeters (Fig. 3).

Figure 4 shows the absolute uptake of the label into the saturated and monounsaturated 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 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 ($R = 0.91$, $p = 0.001$), across all time steps, while the reverse does not hold true ($R = 0.4$, $p = 0.05$). A subsequent pairwise one-way ANOSIM test allocated the 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%). 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
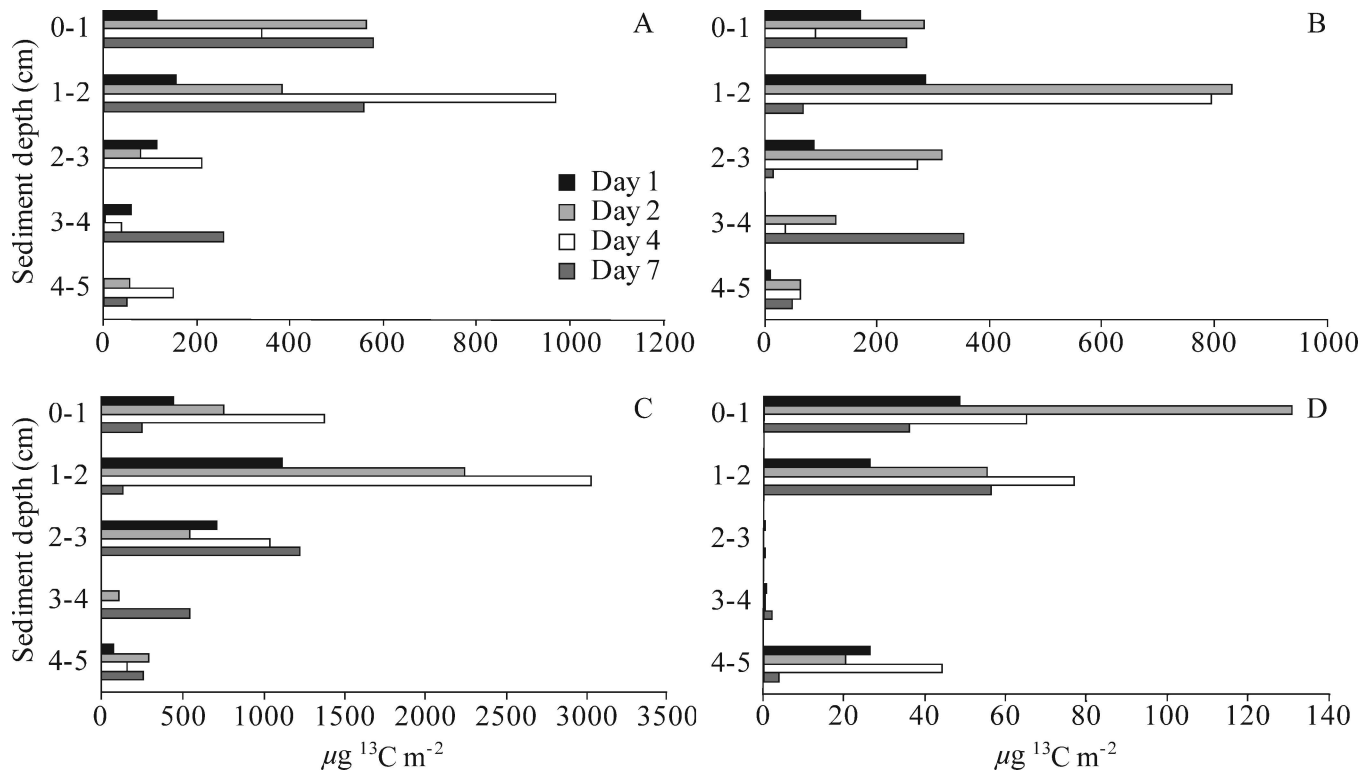
Fig. 3. Total bacterial label incorporation (μg ^{13}C m^{-2}) based on the absolute uptake (I) of the ^{13}C injected substrates over time and depth in the sediment: (A) glucose, (B) amino acids, (C) acetate, and (D) bicarbonate.

(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).

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).

Bacterial carbon contribution to the nematode diet— Natural δ^{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‰ $\pm$ 0.4‰ for 0–1 cm, -19.9‰ $\pm$ 0.8‰ for 1–2 cm, -21.3‰ $\pm$ 2.2‰ for 2–3 cm, -21.9‰ $\pm$ 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. Overall, the $\Delta\delta^{13}$C nematode values were low compared with the

bacterial $\Delta\delta^{13}$C values, suggesting only minute 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 covaried over time (Fig. 6).

Discussion

Given the ubiquity and high abundance of nematodes in deep-sea sediments, it is essential to quantify the nematode community contribution to 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, zooplankton carcasses, fungi, and DOC in the surrounding pore water (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,
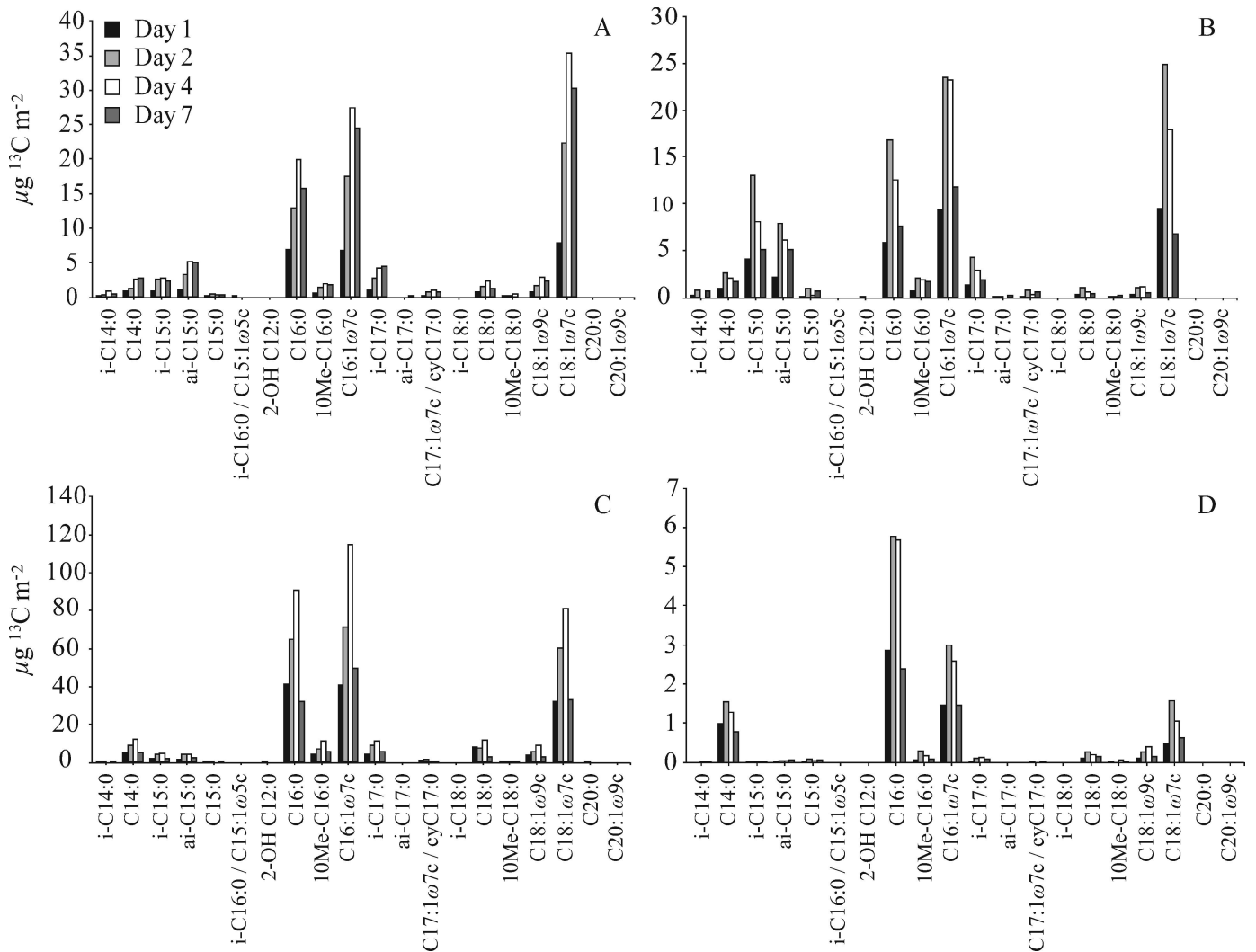
Fig. 4. Absolute uptake (μg ^{13}C m^{-2}) of the ^{13}C injected solutions in bacterial PLFA spectra over time: (A) glucose, (B) amino acids, (C) acetate, and (D) bicarbonate.
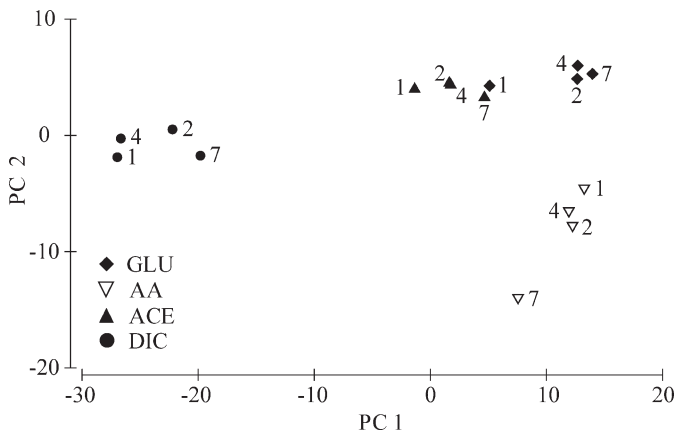

Fig. 5. PCA plot of the bacterial PLFA labeling patterns based on the absolute uptake (I) of the ^{13}C label in the different treatments over time, summed over 0–5-cm sediment depth, with indication of the sampling time step (days 1, 2, 4, and 7). The two PC axes explain 93.1% of the variation observed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

cyanobacteria, and diatoms have been performed in different deep-sea locations but have always resulted in a very low uptake by nematodes (Witte et al. 2003*b*; Moens et al. 2005; Ingels et al. in press (*a* and *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. The sampling location was explicitly chosen to be shallower than the generally suggested limit of around 2000 m, in order to avoid pressure artifacts due to recovery and handling of the
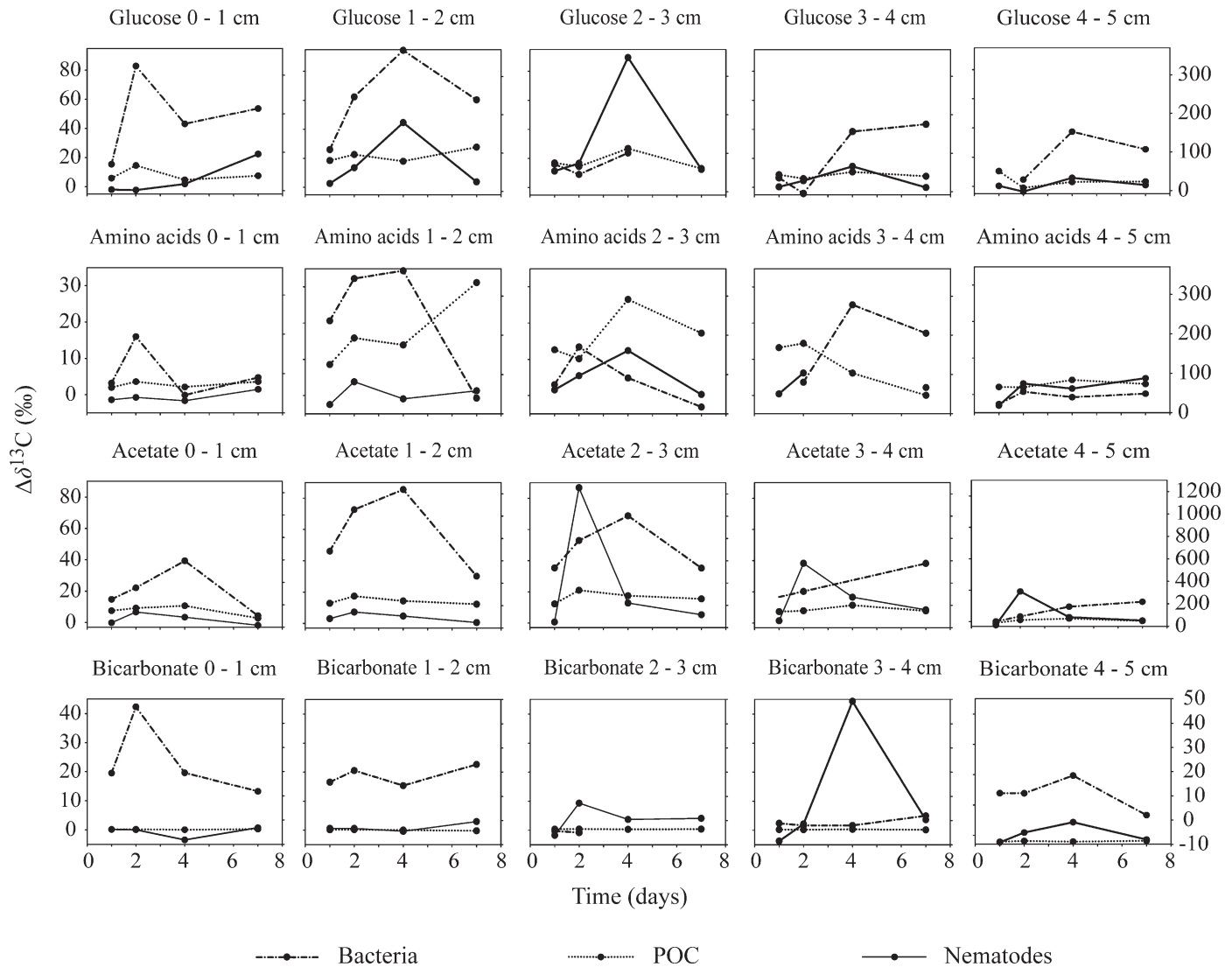
Fig. 6. Measurements over time of the $\Delta\delta^{13}C$ (‰) values of bacteria (right axis), and nematodes and POC (left axis), over the different sediment depths, for the different injection treatments: glucose, amino acids, acetate, bicarbonate.

samples at atmospheric pressure. Woulds et al. (2007) conducted ^{13}C-POM isotope tracer experiments on board and in situ and found substantial faunal uptake for both experimental setups with comparable levels of ^{13}C labeling up to a depth of 940 m. Moreover, most studies in which differences between on board and in situ incubations are reported deal with sediments between 2000 and 4000 m (Woulds et al. 2007), much deeper than our study site. Potentially more problematic than depressurization is the warming of sediment cores during retrieval. However, this warming is considered to be negligible and of no influence on the sediment cores due to the low temperatures of the surface water (4.9°C) and the atmosphere (2.7°C) at the Arctic Hausgarten location at the time of the experiment. 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 toward day 7 suggest high bacterial turnover rates. The immediate and significant labeling of the bacteria can be attributed to the use of simple dissolved organic compounds that were readily accessible in the pore water after injection and that can be transported directly across the bacterial cell wall without being processed by exoenzymes. The highest incorporation of the ^{13}C label into
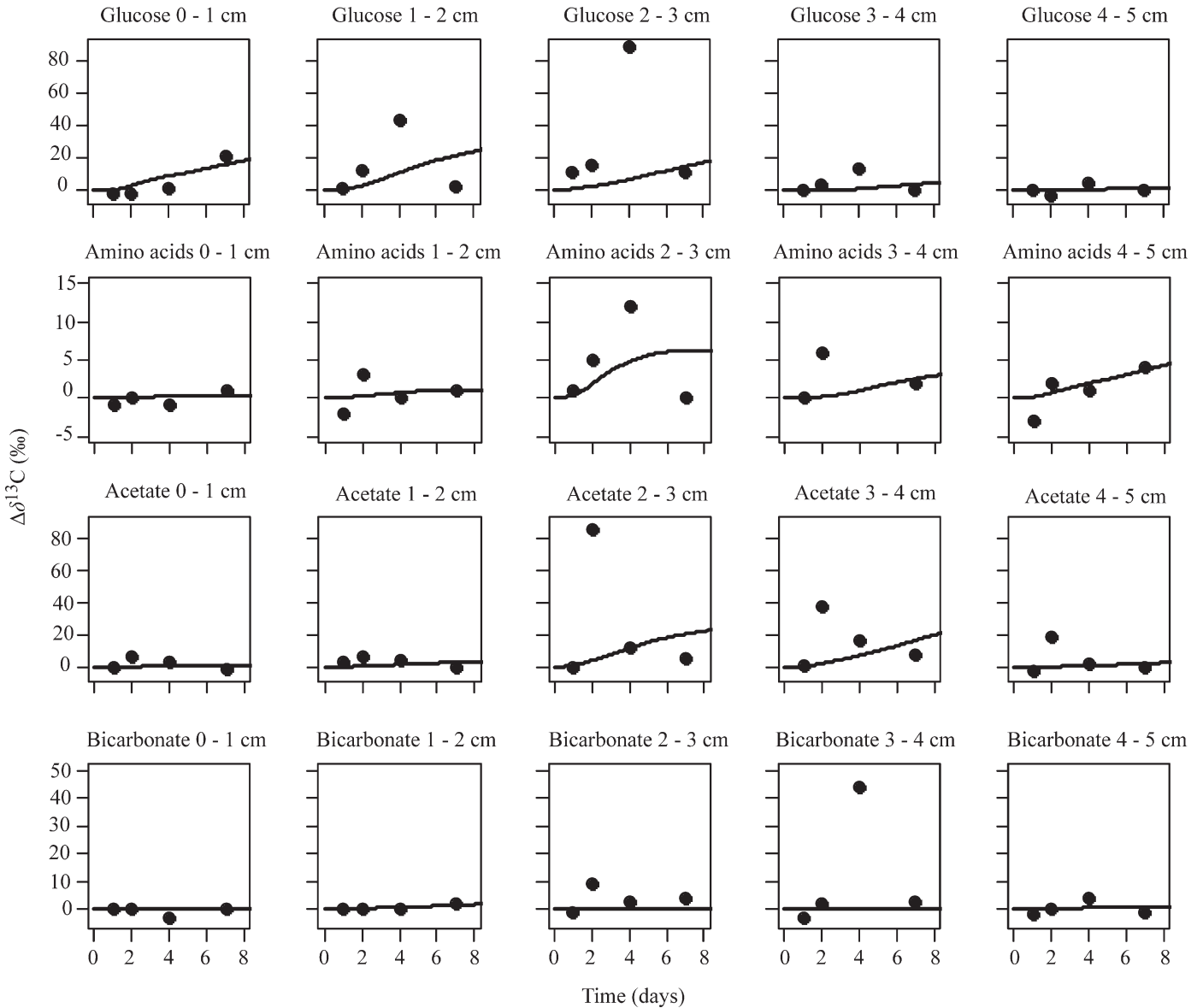
Fig. 7. Measurements over time of the $\Delta\delta^{13}C$ (‰) values of nematodes and the best model fit, over the different sediment depths, for the different injection treatments: glucose, amino acids, acetate, bicarbonate.

bacterial PLFAs took place in the surface 2-cm sediment layers in all experimental treatments. Owing 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 chemoautotrophic bacteria using dissolved inorganic carbon (bicarbonate) and heterotrophic bacteria using dissolved organic carbon substrates (glucose, amino acids, or acetate, Fig. 5). Chemoautotrophic 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 incorporated label significantly 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 that 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-yr study period (2000–2004) (Hoste 2006). There were only minor differences found when the time series was compared with the nematode community from this study. The differences found were mainly due to variations in the relative abundance of genera rather than due to the presence or 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. 2010). *Daptonema* and *Sabatieria* are genera contributing to the similarity ($> 1\%$) between several slope areas worldwide (Vanreusel et al. 2010) 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 imply a maximum presence of 35.4% to 56.2% of bacterivores. Earlier estimates based on mouth morphologies by Jensen (1987) imply that bacteriophageous nematodes make up 70% (de Bovée and Labat 1993) to 90% (Jensen 1988) of the total 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 adequate, the bacterial standing stocks do not appear to be limited, since the nematodes need approximately 0.03–0.22% of the bacterial standing stock to meet their daily respiration requirements. 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 d. 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 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 (1987) 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. (2003*a*), Moens et al. (2005), and Ingels et al. (in press (*a*)) 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. in press (*b*)), 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 dissolved organic matter, by uptake of associated epibiotic bacteria (Ólafsson et al. 1999), 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 a limiting nutrient for benthic organisms (Thomas and Blair 2002), 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. Since 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 $\delta^{13}C$ 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 nanobiotic fungi or more refractory organic matter), preferably on lower nematode taxonomic levels and longer timescales, 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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