

The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an in situ stable isotope labeling study

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Abstract

We report the results of an in situ tracer experiment in an intertidal sediment, where bacterial carbon was tagged with stable carbon–isotope label, after the injection of ^{13}C -glucose. The appearance of label in bacteria (based on label incorporation in bacteria-specific, phospholipid-derived fatty acids) and subsequent transfer to meiobenthos (group level) and macrobenthos (species level) was followed for 36 days. The label dynamics of benthic taxa were either fitted with a simple-isotope model or evaluated against enrichment in bacteria, to derive the importance of bacterially derived carbon for the meiobenthos and macrobenthos. Although selective uptake of bacteria was evident, as 2.4 times more bacterial carbon was grazed as expected from indiscriminate feeding, bacterial carbon accounted on average for only 0.08 and 0.11 of the carbon requirements of meiobenthic and macrobenthic taxa, respectively. Additionally, the contribution of bacterial carbon to total carbon requirements did not depend on the living/feeding depth in the sediment or organism size (evaluated over a size range of four orders of magnitude). The observed overall low contribution of bacterial carbon implies that most intertidal benthic fauna depend primarily on other carbon resources that may assert a stronger control on the structure of intertidal-sediment communities.

Deposit-feeding organisms face the formidable task of gathering digestible resources that are diluted with minerals and refractory organic matter (Lopez and Levinton 1987). Bacteria are ubiquitous in marine sediments, and because of their high abundance, production, and nutritional value, they are considered an important resource for sediment-dwelling fauna (Zobell and Feltham 1937; Gerlach 1978; Tsuchiya and Kurihara 1979).

Transfer of bacterial carbon to benthic fauna is often discussed from the bacterial side: Is bacterial-carbon production a link or sink in the benthic food web (Kemp 1990)? Most studies show that grazing losses are generally restricted to less than 20% of bacterial-carbon production (Kemp 1987; Epstein and Shiaris 1992; Sundback et al. 1996). Although grazing may represent a minor fate of bacterial production, a crucial complementary ecological question is how much the bacterially derived carbon contributes to the total carbon requirements of benthic fauna. However, observations on the trophic significance of bacterial carbon for different benthic taxa are limited. For

some meiobenthos (e.g., nematodes), estimates on the relative importance of bacterial carbon are based on qualitative gut-contents analysis (Moens and Vincx 1997). Quantitative data are available for some macrobenthic deposit feeders that are based on laboratory measurements of sediment-ingestion rate, bacterial abundance, and bacterial-digestion efficiency on the one hand and physiologic data on carbon requirements on the other hand (Cammen 1980; Kemp 1987; Andresen and Kristensen 2002). Most of these studies show that bacterial carbon contributes less than 10% of the total carbon requirements. However, deposit feeders are known for their selective uptake of organic matter (Lopez and Levinton 1987; Neira and Höpner 1994), which may result in ingestion of sediment with higher bacterial abundances, as compared with bulk sediment (Plante and Jumars 1993; Andresen and Kristensen 2002). Therefore, the bacterial contribution as a carbon source may have been underestimated in previous studies that used bulk sediment–bacterial abundance. Moreover, deep-living meiobenthic and subsurface macrobenthic deposit feeders have restricted access to recently produced or deposited labile organic matter, as compared with meiobenthos and macrobenthos living close to the sediment surface. This circumstance may have repercussions on the extent to which they exploit bacteria as a carbon resource, but quantitative data are lacking so far. Quantitative data on the link between bacteria and benthic fauna are essential for understanding the extent to which this trophic link structures sediment communities.

In this paper, we use an in situ stable isotope-labeling method to quantify the importance of bacteria as a carbon source for the meiobenthic and macrobenthic community. ^{13}C -glucose was injected into an intertidal marine sediment to isotopically enrich the bacterial community. Incorporation of ^{13}C -glucose into bacterial carbon was traced through ^{13}C enrichment of bacteria-specific, phospholipid-

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All raw $\Delta\delta^{13}\text{C}$ data presented in this paper will become freely available in the PANGAEA database (<http://www.pangaea.de>) after publication of this manuscript. This is publication 3821 of the Netherlands Institute of Ecology (NIOO-KNAW), Yerseke.

id-derived fatty acids (PLFAs). Subsequent transfer of bacterially derived ^{13}C to benthic fauna was followed through ^{13}C enrichment in hand-picked specimens. A simple-isotope model was used to recover the contribution of bacterial carbon from the observed tracer dynamics. Specifically, we focus on the following questions:

- (1) How much does bacterial carbon contribute to the carbon requirements of meiobenthic groups and macrobenthic species?
- (2) Is the contribution of bacterially derived carbon related to feeding/living depth in the sediment?

Materials and methods

Study site and experimental approach—The data presented here have been collected in the frame of an experiment on the fate of bacterial-carbon production, and two companion papers deal with the fate of bacterial-carbon production (van Oevelen et al. 2006) and the fate of bacterial phospholipids and peptidoglycan (Veuger et al. 2006). The experiment was conducted at the Molenplaat intertidal flat, located in the turbid, heterotrophic, and nutrient-rich Scheldt estuary. The sampling site is located in the silty center of the flat ($51^{\circ}26.25'\text{N}$, $3^{\circ}57.11'\text{E}$), which has a median grain size of $77\text{ }\mu\text{m}$, organic carbon content of approximately 0.5 wt%, and exposure time of about 7 hours per tidal cycle (see Herman et al. 2001 for detailed information).

Methodological details on the experiment are provided in van Oevelen et al. (2006). In short, two 0.25-m^2 metal frames were inserted in the sediment. On 21 May 2003, each experimental plot was labeled by 400 syringe injections into the upper 10 cm of the sediment (i.e., 1 injection per 6.25 cm^2). The syringe was filled with a ^{13}C -glucose solution and gradually emptied during retraction from the sediment to achieve a uniform depth distribution of the label. Labeling was performed daily for 5 consecutive days to ensure sufficient label incorporation by bacteria, but labeling on day 2 was canceled because of bad weather. The resulting ^{13}C flux amounted to $15.3\text{ mmol of }^{13}\text{C m}^{-2}$ per labeling day. Ten samples (days 0.3, 2, 3, 4, 5, 6, 8, 12, 18, and 36 after the first injection) were collected from each plot in the first weeks from a priori randomly assigned positions. A sampling core (inside diameter 5 cm) was inserted 10 cm deep, filled with filtered sea water, and closed with a stopper. A metal core (inside diameter 9 cm) was inserted around the sampling core, which successfully prevented disturbance of the rest of the plot and remained in place during the experiment. The sampling core was carefully withdrawn and transported in a dark, cool container to the laboratory. In the laboratory, sediment cores were sliced (0–2, 2–5, and 5–10 cm), homogenized, and sampled for $\delta^{13}\text{C}$ -PLFA, meiobenthic biomass, and label incorporation and macrobenthic label incorporation. Samples for $\delta^{13}\text{C}$ -PLFA were frozen, freeze-dried, and stored frozen. Samples for meiobenthos and macrobenthos were fixed with formalin (final concentration 4%). Background $\delta^{13}\text{C}$ for PLFA, meiobenthos, and macrobenthos were taken

from the $t = 0$ sampling core. Some macrobenthic species were not present in the $t = 0$ samples, in which cases background $\delta^{13}\text{C}$ values were taken from Herman et al. (2000). Macrobenthic biomass could not be accurately determined from the small cores taken from the experimental plots and was, therefore, based on 12 separate cores (inside diameter 10 cm) taken in close proximity to the experimental plots.

Analytical procedures—Lipids were extracted from 3 g of dry sediment by use 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–isotope ratio mass spectrometry (GC-IRMS) for PLFA isotope values (details in Middelburg et al. 2000). The bacterial-isotope signature was determined from the weighted average of bacteria-specific PLFA biomarkers i14:0, i15:0, a15:0, i16:0, and 18:1 ω 7c. PLFAs are present in the membrane and comprise roughly 6% of the total carbon in a bacterial cell; the bacteria-specific PLFAs together account for 28% of the carbon in all bacterial PLFAs. These conversion factors were used to convert PLFA concentration to bacterial biomass and label incorporation in PLFAs to total-bacterial label incorporation (Middelburg et al. 2000).

Meiobenthic samples were sieved ($38\text{ }\mu\text{m}$) and subsampled. Specimens for stable isotope measurements, typically 15 to 30 specimens of each individual meiobenthic group, were hand-picked, cleaned of adhering detritus, rinsed ($0.2\text{-}\mu\text{m}$ filtered water), transferred to silver boats, and stored frozen. Processing of the meiobenthic samples proved to be very time consuming, and only one of the two plots was therefore processed for stable isotope and biomass data. See Moodley et al. (2000) for processing details.

Macrobenthic specimens were hand-picked, and the sorted sample was transferred to a petri dish, after which individual species were taken, cleaned of debris, rinsed, transferred to a silver boat, and stored frozen. Bivalves and gastropods were placed in an acidified bath (1 mmol HCl) to dissolve their carbonate shell, and either whole specimens (*Macoma balthica* [$<7\text{ mm}$]) and *Hydrobia ulvae*) or flesh samples (*M. balthica* [$\geq 7\text{ mm}$]) were taken. Finally, meiobenthic and macrobenthic samples were acidified for carbonate removal with $20\text{ }\mu\text{L}$ of 2.5% HCl and oven dried (50°C) before isotope analysis. Stable-isotope ratios were measured by elemental analyzer–isotope ratio mass spectrometry (EA-IRMS) (Middelburg et al. 2000).

Delta values are expressed relative to the carbon-isotope ratio ($R = ^{13}\text{C} : ^{12}\text{C}$) of Vienna Pee Dee Belemnite (VPDB): $\delta^{13}\text{C} = (R_{\text{sample}} : R_{\text{VPDB}} - 1) \times 1000$, with $R_{\text{VPDB}} = 0.0112372$. Label uptake is reflected as enrichment in ^{13}C and is presented as $\Delta\delta^{13}\text{C}$ (‰), which indicates the increase in $\delta^{13}\text{C}$ of the sample, as compared with its natural ground value, and is calculated as $\Delta\delta^{13}\text{C}$ (‰) = $\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{background}}$. Hence, positive $\Delta\delta^{13}\text{C}$ values indicate that the organism has acquired some of the introduced label.

Tracer model and calibration—The relative contribution of bacterially derived carbon was estimated from comparison of the $\Delta\delta^{13}\text{C}$ of a consumer ($\Delta\delta^{13}\text{C}_{\text{con}}$) with that of

bacteria ($\Delta\delta^{13}\text{C}_{\text{bac}}$). If the $\Delta\delta^{13}\text{C}_{\text{bac}}$ and $\Delta\delta^{13}\text{C}_{\text{con}}$ have reached steady-state, the ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ indicates the fraction of total carbon in the consumer that is derived from bacteria. However, this steady-state assumption is not valid for all organisms within an experimental time frame of several weeks (Hall and Meyer 1998), and an isotope model that simulates tracer dynamics in a consumer is then a better option (Hamilton et al. 2004). However, the isotope model can only be applied for frequently sampled species because of its higher data requirements. Therefore, the contribution of bacterial carbon for those species that were encountered repeatedly in the time-series samples was estimated by means of the isotope model. For species that were only occasionally encountered, contribution of bacterially derived carbon was estimated from the ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$.

The isotope model reads (Hamilton et al. 2004)

$$\frac{d\Delta\delta^{13}\text{C}_{\text{con}}}{dt} = k_b \cdot \Delta\delta^{13}\text{C}_{\text{bac}} - k_c \cdot \Delta\delta^{13}\text{C}_{\text{con}} \quad (1)$$

The first term in the right-hand side of the equation denotes label uptake by grazing on bacteria, and the second term denotes label loss through turnover of the consumer. The dynamic of bacterial PLFAs are used as a proxy for $\Delta\delta^{13}\text{C}_{\text{bac}}$ data and are imposed as a forcing function. The turnover-rate constant (k_c) determines the total carbon requirements of the consumer, and the ratio k_b/k_c denotes the relative contribution of bacterial carbon to total carbon requirements. If bacteria fulfill total carbon requirements (i.e., $k_b/k_c = 1$), the $\Delta\delta^{13}\text{C}$ of the consumer approaches that of bacteria with time, and when bacteria do not contribute (i.e., $k_b = 0$), no label uptake by the consumer occurs (see Hamilton et al. 2004 for model demonstrations).

Plausible parameter ranges were chosen large to assure a complete coverage of potential growth rates and were $0.05\text{--}0.50\text{ d}^{-1}$ (k_c) and $0.0\text{--}0.50\text{ d}^{-1}$ (k_b) for meiobenthos and $0.025\text{--}0.25\text{ d}^{-1}$ (k_c) and $0.0\text{--}0.25\text{ d}^{-1}$ (k_b) for macrobenthos. The parameters were calibrated by minimization of the sum of squared differences between the data points and the model prediction.

Calibration of the model parameters k_b and k_c individually was not possible, as different combinations gave similar optimal fits, which indicates that the parameters are correlated. To resolve this issue, we employed Bayesian analysis. Bayesian analysis is a statistical technique that updates a prior probability distribution of a parameter with observations to arrive at a posterior probability distribution (Gelman et al. 2003). The update makes the posterior better constrained than the prior. The prior probability distributions are the initial parameter ranges for which we assume equal probability for each value within this range. The Bayesian analysis starts with a model run with a certain parameter combination, and a Markov chain Monte Carlo technique (Gilks et al. 1998) then takes random steps in parameter space with which the model is solved. If a parameter combination gives a better fit to the data than

does the previous parameter combination, the run is accepted and used as new starting point for a following random step. If the new parameter combination fits worse, it can be accepted with a probability equal to the ratio of probabilities of the tested versus the existing parameter combination. The distribution of parameter values in the set of accepted runs is the posterior probability distribution of each parameter. We ran the model for each species 10,000 times, which typically gave approximately 1,000 accepted runs. The mean and standard deviation of the ratio k_b/k_c for a species was then calculated from the accepted model runs. The model was implemented in the freely available simulation environment FEMME (Soetaert et al. 2002, <http://www.nioo.knaw.nl/ceme/femme>).

To test whether the contribution of bacterial carbon for meiobenthos increases with depth in the sediment, the data of a meiobenthic group of a respective depth interval was fitted with the bacterial $\Delta\delta^{13}\text{C}$ of that respective depth interval. For macrobenthos, directly linking presence in a certain depth interval with feeding depth is difficult because the size of these species ($\sim\text{cm}$) is comparable to that of the depth intervals. Therefore, we used the feeding classification for macrobenthic species (Fauchald and Jumars 1979) and compared surface deposit and subsurface deposit feeders. When appropriate, data from all depth layers were pooled and used to calculate the contribution of bacterial carbon for meiobenthic and macrobenthic taxa.

Results

Benthic biomass—Bacterial biomass, determined from concentrations of bacteria-specific PLFAs, summed over the 10-cm depth interval and averaged over the experimental period, was 781 mmol C m^{-2} (van Oevelen et al. 2006). Meiobenthic biomass was 188 mmol C m^{-2} and was dominated by nematodes (35%), hard-shelled foraminifera (33%), and juveniles of the polychaete *Heteromastus filiformis* (18%) (Table 1). Copepods, soft-bodied foraminifera, other juvenile polychaetes, and turbellaria each comprised 5% or less of the meiobenthic biomass. The meiobenthic biomass was highest in the top interval (0–2 cm, 72%), and all meiobenthic groups were present here (Table 1). The number of meiobenthic groups decreased with depth, with only nematodes and hard-shelled and soft-bodied foraminifera present in the deepest layer. Nematodes dominated biomass in the top two intervals (37% and 58%, respectively), whereas hard-shelled foraminifera clearly dominated in the deepest interval (91%) (Table 1). *H. filiformis* juveniles represented a significant amount of the meiobenthic-sized biomass in the top layer (24%), but their biomass vanished in the middle-depth interval.

Macrobenthic biomass was $1,684\text{ mmol C m}^{-2}$, and label uptake was measured in species that represented 96% of the biomass (Table 1), albeit with different frequencies. The remaining 4% of the biomass was made up of species that were not sampled with the cores taken from the experimental plots. Large specimens of the bivalve *Macoma*

Table 1. Contribution of bacterial carbon to total carbon requirements for meiobenthos (group level) and macrobenthos (species level).

Species/group	Biomass	d1	d2	d3	FM	$\frac{k_b}{k_c}$	$\frac{\Delta\delta^{13}C_{con}}{\Delta\delta^{13}C_{bac}}(\text{day})$
Copepods	3	2	0.1	—		0.06±0.02	0.03 (36)
Hard-shelled foraminifera	62	31	6	25		0.09±0.03	0.02 (36)
Soft-bodied foraminifera	5	4	0.5	0.6		0.14±0.04	0.03 (36)
Juvenile <i>H. filiformis</i>	34	32	2	—		0.14±0.03	0.05 (36)
Nematodes	67	50	15	2		0.06±0.02	0.09 (36)
Juvenile polychaetes	4	4	0.2	—		0.03±0.01	0.02 (36)
Turbellaria	4	4	—	—			0.14 (18)
Unknown species	9	7	2	—			0.02 (36)
<i>Corophium</i> spp.	4	3	1	0.2	SDF	0.12±0.05	0.00 (36)
<i>Eteone</i> spp.	15	2	13	0.3	P	0.15±0.04	0.11 (36)
<i>H. filiformis</i>	597	10	194	393	SSDF	0.21±0.05	0.30 (36)
<i>M. balthica</i> (<7 mm)	28	28	0.2	—	SDF	0.36±0.09	0.05 (36)
<i>M. balthica</i> (≥7 mm)	671	—	612	59	SDF	0.11±0.04	0.16 (36)
<i>P. cornuta</i>	34	33	0.5	—	SF/SDF	0.20±0.04	0.10 (36)
<i>P. elegans</i>	215	185	30	0.1	SDF	0.15±0.03	0.14 (36)
<i>A. marina</i>	0.2	0.09	0.1	—	SSDF		0.23 (8)
<i>C. carinata</i>	7	3	4	0.2	P		0.00 (6)
<i>H. ulvae</i>	24	24	—	0.2	SDF		0.01 (18)
<i>Nereis</i> spp.	21	0.3	—	21	O		0.05 (36)
<i>S. benedicti</i>	1	0.2	0.8	—	SDF		0.01 (18)
<i>T. marioni</i>	3	0.3	2	—	SDF		0.06 (36)

Biomass (mmol C m⁻²) is shown as depth integrated and partitioned over the three depth intervals (d1: 0–2 cm, d2: 2–5 cm, d3: 5–10 cm). Feeding modes (FM) for macrobenthos are surface-deposit feeder (SDF), predator (P), subsurface-deposit feeder (SSDF), suspension feeder (SF), or omnivore (O). The ratio k_b/k_c is the contribution of bacterial carbon to total carbon demands presented as average ± standard deviations and derived from the isotope model (see Materials and methods). Numbers in italic indicate a poor model fit (see Discussion). The ratio $\Delta\delta^{13}C_{con}/\Delta\delta^{13}C_{bac}$, with the day number of sampling in parentheses, is shown for all sampled meiobenthos and macrobenthos.

balthica (≥7 mm, 41%) and the polychaetes *Heteromastus filiformis* (37%) and *Pygospio elegans* (13%) dominated the macrobenthic biomass (Table 1). Macrobenthic biomass did not show a pronounced trend with depth in the sediment, because the dominant species *M. balthica* (≥7 mm) and *H. filiformis* have their biomass maxima in the middle-depth and deepest-depth intervals, respectively. This presence compensates the strong decrease in biomass with depth of other species, such as *P. elegans*, *Polydora cornuta*, *M. balthica* (<7 mm), *Hydrobia ulvae*, and *Corophium* spp., which all had more than 80% of their biomass in the top layer. The species *Arenicola marina*, *Eteone* spp., and *Cyatura carinata* had their highest biomass in the middle-depth interval.

Bacterial label incorporation—The $\Delta\delta^{13}C$ of different bacterial PLFAs were weighted with their respective concentration to obtain a proxy for bacterial $\Delta\delta^{13}C$. Dynamics of bacterial $\Delta\delta^{13}C$ was very consistent between plots and intervals for the upper two depth intervals (0–2 and 2–5 cm), but the deepest interval (5–10 cm) showed differences between the plots and had somewhat higher bacterial $\Delta\delta^{13}C$ values than did the upper two intervals (Fig. 1A). Because of the lower concentration of PLFA in deeper intervals (374, 181, 227 mmol C m⁻² in depth intervals 0–2, 2–5, and 5–10 cm, respectively), their influence on the weighted bacterial $\Delta\delta^{13}C$ is limited (Fig. 1A). The average forcing function, weighted with concentration and thickness of the depth interval, for

bacterial $\Delta\delta^{13}C$ was used in the isotope model or ratio calculations because the results did not depend critically on whether distinctions were made among intervals or plots (see Discussion). The bacterial $\Delta\delta^{13}C$ increased during and shortly after the ¹³C-glucose injection period, peaked at 519‰ on day 5, and decreased to 173‰ at day 36.

Meiobenthic and macrobenthic label incorporation—All sampled species acquired ¹³C label, but $\Delta\delta^{13}C$ dynamics differed among groups and species (Fig. 1). Among the meiobenthos, juvenile polychaetes (Fig. 1N), and copepods (Fig. 1I) only marginally increased in $\Delta\delta^{13}C$ and remained below 20‰ during the experiment. Juvenile *Heteromastus filiformis* and soft-bodied and hard-shelled foraminifera (Fig. 1L,K, J) showed similar label dynamics, with a steady increase to 60‰, followed by an exponential-like decrease to almost background levels at day 36.

Among the macrobenthos, *Macoma balthica* (<7 mm [Fig. 1E]) attained highest $\Delta\delta^{13}C$ values (217‰ at day 7) but almost returned to background values at day 18 (22‰). In contrast, *Heteromastus filiformis* incorporated label slowly, and its $\Delta\delta^{13}C$ remained constant at approximately 50‰ over a month (Fig. 1D). Labeling of large *M. balthica* (≥7 mm [Fig. 1F]) specimens was highly variable but overall lower than labeling of small specimens (Fig. 1E). Label incorporation by *Corophium* spp. was very rapid and peaked at 82‰, but its $\Delta\delta^{13}C$ signal rapidly decreased in an exponential fashion when the ¹³C-glucose injection had ended (Fig. 1B). The $\Delta\delta^{13}C$ dynamics of polychaetes

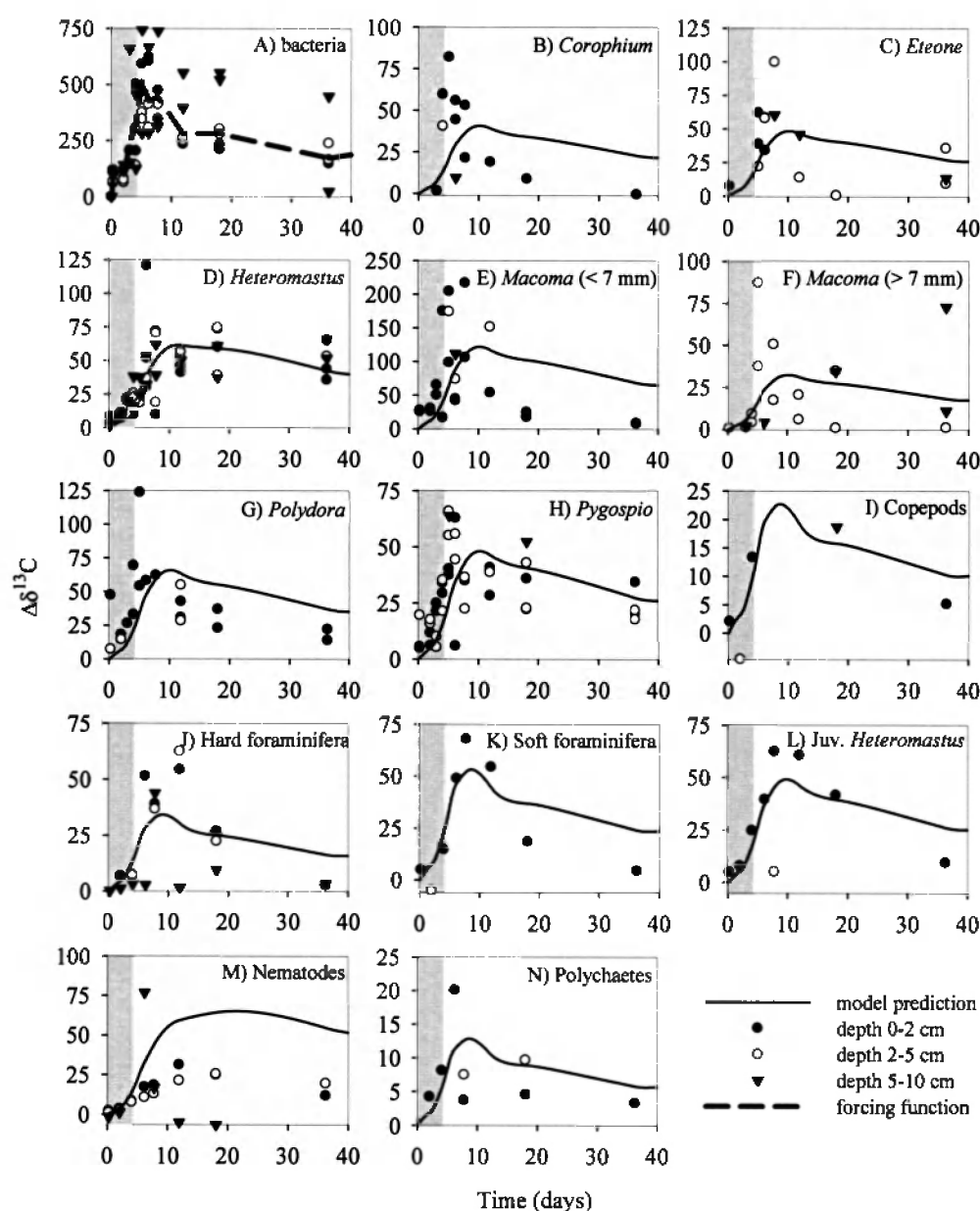


Fig. 1. (A) Observations and forcing function of bacterial $\Delta\delta^{13}\text{C}$ (‰). Observations and best fit for the macrobenthic species (B) *Corophium* spp. ($n = 12$), (C) *Eteone* spp. ($n = 15$), (D) *Heteromastus filiformis* ($n = 58$), (E) *Macoma balthica* (< 7 mm) ($n = 23$), (F) *M. balthica* (≥ 7 mm) ($n = 18$), (G) *Polydora cornuta* ($n = 19$), (H) *Pygospio elegans* ($n = 41$), and meiobenthic groups (I) copepods ($n = 5$), (J) hard-shelled foraminifera ($n = 20$), (K) soft-bodied foraminifera ($n = 8$), (L) juvenile *H. filiformis* ($n = 9$), (M) nematodes ($n = 22$), and (N) juvenile polychaetes ($n = 8$). Shown are data pooled from both plots in the three depth intervals. Shaded area indicates period of ^{13}C -glucose injection.

Polydora cornuta and *Pygospio elegans* resemble each other with a steady increase during the first 10 days, followed by a slow decrease (Fig. 1G,H).

Contribution of bacterial carbon—As pointed out earlier, fitting the individual parameters k_b and k_c of the isotope model proved impossible because of a correlation between these parameters. For example, many combinations gave an acceptable fit of the observed $\Delta\delta^{13}\text{C}$ values for the

polychaete *Heteromastus filiformis* (Figs. 1D, 2A). This feature was observed for all groups and species analyzed with the isotope model. However, the relative contribution of bacterial carbon to total carbon requirements (i.e., k_b/k_c) has our prime interest, and this ratio is much better constrained than the individual parameters (Fig. 2A). The distribution of the ratios in the accepted set of Bayesian runs approximates a normal distribution, from which the average and standard deviation can be derived (Fig. 2B).

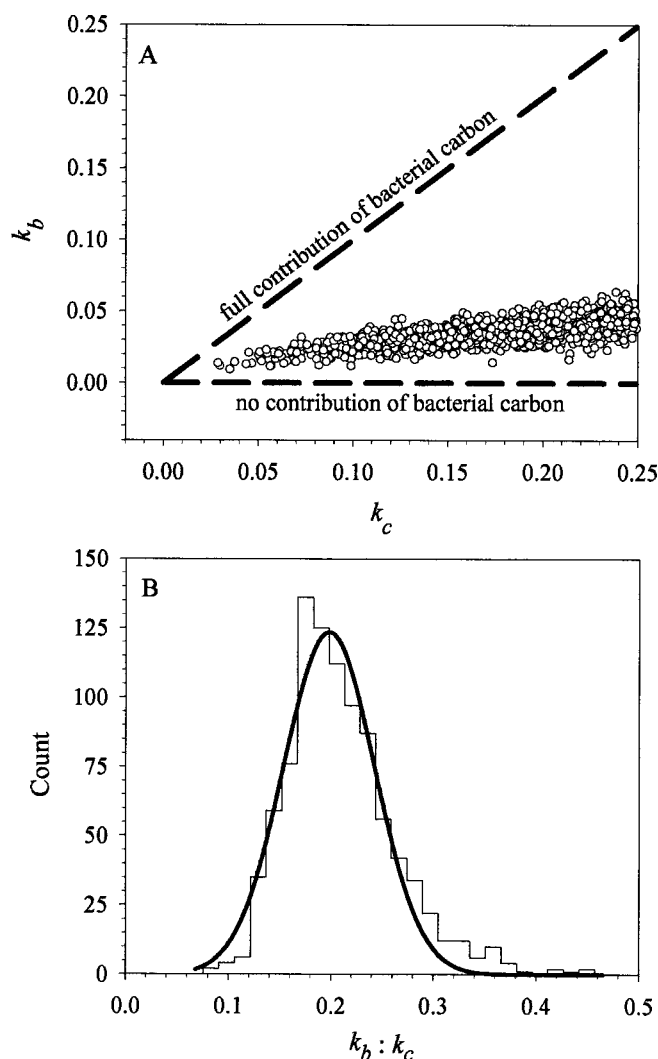


Fig. 2. (A) Scatter plot of the accepted Bayesian runs of the model parameters k_b against k_c for *Heteromastus filiformis*. The linear relation among both parameters shows that the ratio of the parameters is better constrained than the individual parameter values. (B) Histogram of the ratio k_b/k_c from the data in panel A and the fitted normal distribution. Histograms for other organisms fit the normal distribution equally or better.

The histograms for the other species/groups are not shown, but all gave a similar picture. The $\Delta\delta^{13}\text{C}$ dynamics of most consumers could be readily fitted with the simple isotope-turnover model (Fig. 1). Good visual fits were, however, not obtained for *Macoma balthica*, in particular the small (<7 mm) specimens (Fig. 1E), and *Corophium* spp. (Fig. 1B), for which peak labeling and label-loss rate were underestimated by the model.

Both the results from the isotope model (Eq. 1) and the results from ratio estimates $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ show that the contribution of bacterial carbon was limited (Table 1). The contribution of bacterial carbon to total carbon requirements for all meiobenthic groups was 0.14 or less and averaged 0.08. The estimates for macrobenthos were more variable and ranged from 0.00 to 0.23, but averaged 0.11 (Table 1). The contribution of bacterial carbon to

small *M. balthica* (<7 mm) was higher (0.36), but we consider this estimate unreliable because of the poor fit to the data, which possibly indicates direct uptake of ^{13}C -glucose (see Discussion). Despite the variability in the $\Delta\delta^{13}\text{C}$ data, bacterial-carbon contributions to diets could be readily estimated for most species, as most standard deviations are within 25% to 30% of the mean.

Nematodes and hard-shelled foraminifera were encountered frequently enough in all depth-interval samples to use the isotope model to examine whether the contribution of bacterial carbon changed with depth in the sediment. Nematodes did not show differences with regard to living depth (0.08 ± 0.02 , 0.08 ± 0.02 , and 0.06 ± 0.04 for 0–2, 2–5, and 5–10 cm, respectively). The contribution of bacterial carbon for hard-shelled foraminifera was similar for the upper two sediment layers (0.13 ± 0.03 and 0.15 ± 0.05) but was much lower in the deepest sediment layer (0.03 ± 0.02), where they dominated meiobenthic biomass. Other meiobenthic groups had three or fewer observations in the middle or deepest depth layer, which does not allow reliable fitting with the isotope model. However, the ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ of all samples was smaller or similar to the estimated contribution of bacterial carbon estimated for the top sediment layer for each group (data not shown), which indicates no increased contribution with depth for these meiobenthic groups.

No large differences occurred in the contribution of bacterial carbon among macrobenthic feeding modes (Table 1), although the contribution of bacterial carbon was slightly higher for the subsurface feeders *Heteromastus filiformis* (0.21) and *Arenicola marina* (0.23), as compared with surface feeders (typically between 0.10 and 0.15 [Table 1]). When the ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ of all macrobenthic species was evaluated in each depth layer, it showed no trend in a change in contribution of bacterial carbon with respect to sediment depth (data not shown).

Discussion

Sediment organic matter is a complex mixture of pools that differ in lability, nutritional value, and origin, and linking carbon sources to the sediment-dwelling community in situ is very difficult. We have successfully employed a stable isotope-labeling approach to quantify the importance of bacteria as a carbon source for most members of the meiobenthos and macrobenthos of an intertidal-flat sediment community.

Before moving to the implications of our results, we address some points that might complicate our interpretations. $\Delta\delta^{13}\text{C}$ -labeling patterns of bacteria were very consistent between the two plots for the upper two depths but were more variable for the deepest-depth layer (Fig. 1A). The average bacterial $\Delta\delta^{13}\text{C}$ captures the overall dynamics well, particularly for the upper two depths, where most of the grazing has taken place. Nevertheless, labeling of some grazers was rather variable (Fig. 1), although at a level that is typical for in situ labeling experiments (Hall and Meyer 1998; Herman et al. 2000; Middelburg et al. 2000). To examine the effect of this variability on our results, the observations of the frequently sampled macro-

benthic species *Heteromastus filiformis* were fitted separately for each plot, with the bacterial $\Delta\delta^{13}\text{C}$ for the respective plot as forcing function. The derived contributions were similar for both plots (0.21 ± 0.04 versus 0.21 ± 0.06). Because other species have a similar level of variability, we assert that our results are robust, despite the high variability inherent in this type of experiment.

Alternative label pathways—On average, no more than 0.10 to 0.15 of the total carbon demands of benthic fauna was met by bacterial carbon (Table 1). This amount might have even been lower if fauna acquired ^{13}C label through other pathways than grazing on bacteria. Although alternative routes of label transfer may exist in intertidal sediments, we expect none to have seriously obscured the outcome of the experiment.

First, benthic organisms may have acquired label by direct utilization of the injected ^{13}C -glucose, which would result in an overestimation of the contribution of bacterial carbon. The timescale of the experiment (\sim weeks) is much longer than the turnover time of glucose (\sim minutes [Sawyer and King 1993]) but comparable with that of bacteria (\sim weeks [Schallenberg and Kalff 1993]). Consequently, direct ^{13}C -glucose uptake would be characterized by immediate labeling of fauna that stops directly after the injection period, whereas uptake of ^{13}C -labeled bacteria would be characterized by a delayed and longer-lasting uptake of label. Among the benthic fauna, only small *Macoma balthica* (<7 mm) and *Corophium* spp. showed rapid labeling, with peak labeling shortly after the ending of the ^{13}C -glucose injection and a very rapid loss of label (Fig. 1B,E), which cannot be reconciled by bacterial grazing alone. Consequently, the data could not be fitted with the bacterial $\Delta\delta^{13}\text{C}$ dynamics as forcing function (Fig. 1B,E), which again suggests alternative routes of label uptake. For these reasons, we regard the estimates for *M. balthica* (<7 mm) and *Corophium* spp. as unreliable, because they may have utilized dissolved organic matter (DOC). Because data on $\Delta\delta^{13}\text{C}$ of DOC are not available, we cannot evaluate the importance of this carbon source. Most species however, continued to take up label in the first days after completion of ^{13}C -glucose injection, and some $\Delta\delta^{13}\text{C}$ trajectories approached a rather constant level (Fig. 1), both of which are indications of label uptake through grazing on labeled bacteria. Moreover, model simulations explicitly taking DOC uptake into account revealed that total meiobenthic and macrobenthic communities derive 0.10 and 0.20, respectively, of their total carbon demands from bacteria (van Oevelen et al. 2006), consistent with the results presented here for the individual groups/species. The ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ used to quantify the contribution of bacterial carbon for infrequently sampled groups/species is a valid approach only at or close to steady state. For these groups/species, we cannot assess possible inference of ^{13}C -glucose uptake or whether or not steady state has been reached, and these estimates should, therefore, be viewed with more caution than estimates from the isotope model. Nevertheless, these ratio estimates are in general agreement with the estimates from the isotope model (Table 1).

Second, a potential complication is uptake of ^{13}C -glucose by bacteria attached to the fauna body surface (i.e., epicuticular bacteria), a potential artifact that was reported for ^{14}C -acetate in copepods bacterivory experiments (Carman 1990). However, to explain a $\Delta\delta^{13}\text{C}$ of 50‰ (a typical value for benthos [Fig. 1]), and assuming that epicuticular bacteria have a $\Delta\delta^{13}\text{C}$ similar as sedimentary bacteria (280–519‰), thoroughly cleaned meiobenthic and macrobenthic specimens would have consisted of 11% to 22% of epicuticular bacteria. Therefore, although epicuticular bacteria might elevate the $\Delta\delta^{13}\text{C}$ signal of consumers, this is unlikely to explain the major part of the signal. Moreover, arguments with regard to the short turnover time of ^{13}C -glucose, as given above, also apply here.

Third, we consider label transfer from the diatom-dominated microphytobenthos to benthic fauna. Microphytobenthos may be labeled either by direct uptake of ^{13}C -glucose or, more likely, by fixation of inorganic ^{13}C that has been produced during respiration of ^{13}C -glucose. However, ^{13}C enrichment in the PLFA biomarker of diatoms (C20:5 ω 3 [Middelburg et al. 2000]) only significantly increased in the top layer of the sediment and was lower ($\Delta\delta^{13}\text{C}$ of C20:5 ω 3 less than 25‰ during first 6 days, with maximum of approximately 40‰) than that of most benthic fauna grazers and much lower than isotope enrichment of bacteria (Fig. 1). Moreover, the increase in $\Delta\delta^{13}\text{C}$ of C20:5 ω 3 showed a clear time lag, which was not observed in the $\Delta\delta^{13}\text{C}$ dynamics of benthic fauna (Fig. 1). Accordingly, transfer of ^{13}C to fauna via microphytobenthos was low and did not constitute an important alternative pathway of ^{13}C label. Finally, predators and omnivores grazing on other fauna may have acquired ^{13}C label indirectly.

Although not considered a major artifact, alternative pathways would have resulted in an overestimation of the contribution of bacterial carbon and further strengthen the general trend of a limited importance of bacteria as a carbon source for intertidal meiobenthos and macrobenthos.

Bacteria as a carbon source—Because of methodologic difficulties in measurement of bacterivory and total carbon-demand requirements simultaneously, few studies have quantified the relative importance of bacterial carbon for benthic fauna. Sundback et al. (1996) measured grazing rates on microphytobenthos and bacteria by the meiobenthic groups nematodes, harpacticoids, and “others” in a microtidal sandy sediment. Grazing on microphytobenthos exceeded that on bacteria to the extent that the contribution of bacterial carbon was generally restricted to less than 10%. Our results agree with these estimates, especially for nematodes and “other” meiobenthic groups. The nematode community at our study site was found to be dominated by *Tripyloides gracilis*, *Viscosia viscosa*, *Ptycholaimellus ponticus*, and *Daptonema tenuispiculum* (Steyaert et al. 2003). Moens and Vincx (1997) used gut-contents analysis to identify particulate food sources of estuarine nematodes and found that bacterial carbon was of limited importance for these species. The contribution of bacterial carbon for nematodes was 0.06 ± 0.02 (Table 1) and

corroborates the results from gut-contents analysis. They suggested that DOC uptake may potentially be important for some nematodes. This observation was, however, not evident in our study because of the low and delayed labeling of nematodes (Fig. 1M).

Among the permanent meiobenthic groups, highest contribution of bacterial carbon was found for hard-shelled and soft-bodied foraminifera (0.09 and 0.14, respectively [Table 1]). These protozoans gather food particles through a network of pseudopodia and can actively select particles before they are ingested (Moodley et al. 2000). The selected nutritious particles might be highly populated by bacteria, which could explain the relatively high contribution of bacterial carbon.

The majority of estimates on the importance of bacteria as a carbon source concern macrobenthic deposit feeders and are based on measured sediment-ingestion rate and bacterial abundance in relation to carbon requirements assessed from physiologic measurements or literature data (Cammen 1980; Kemp 1987; Cheng and Lopez 1991). Cammen (1980) found that bacteria supplied between 7% and 10% of the carbon requirements of the deposit feeder *Nereis succinea*. Because of fragmentation of the specimens, we were unable to distinguish between *N. diversicolor* and *N. succinea*, but the ratio $\Delta\delta^{13}\text{C}_{\text{con}}/\Delta\delta^{13}\text{C}_{\text{bac}}$ of 0.05 (day 36) confirms the limited contribution of bacterial carbon for *Nereis* (*Hediste*) spp. (Table 1).

Arenicola marina exhibits strong bacteriolytic activity in its midgut section (Plante and Mayer 1994), which reduces the ambient bacterial density up to 70% during transition of the digestive tract (Grossmann and Reichardt 1991) and suggests a strong contribution of bacterial carbon. However, gut-contents analysis and subsequent carbon-budget calculations show that bacteria fulfill only 0.03 to 0.08 of the total carbon requirements of *A. marina* (Andresen and Kristensen 2002). Because of the low density of *A. marina* at our study site, we obtained only one $\Delta\delta^{13}\text{C}$ observation (day 8), from which we estimate a contribution of bacterial carbon of 0.23 (Table 1). This figure should be taken with caution because of limited sampling, but it suggests that bacteria may sometimes be a more important carbon source. Application of the labeling approach in areas densely populated with *A. marina* to examine in situ the importance of bacterial carbon would be interesting.

Clough and Lopez (1993) investigated the importance of potential carbon sources for *Heteromastus filiformis*. Bacterial carbon was not considered important, because only 26% of the ingested bacteria were assimilated during gut passage. This figure alone is not sufficient to quantify the contribution of bacterial carbon, because the ingestion rate of bacterial carbon is also required. By following their budget calculations for organic matter based on indiscriminate feeding and assuming that bacterial carbon is 1% of sedimentary organic carbon, we arrive at a contribution of approximately 3% in the budget of *H. filiformis*. This amount is much lower than our estimate of 21% (Table 1). However, after sediment ingestion and passage through the digestive tract, the fecal casts of *H. filiformis* are usually still several times enriched in organic carbon, nitrogen, and

protein content relative to the bulk sediment at feeding depth, which clearly shows selective feeding capabilities of *H. filiformis* (Neira and Höpner 1994; Wild et al. 2005). If these worms select preferentially reactive organic matter with high bacterial abundance, this selection could account for the observed high contribution of bacterial carbon, as compared with the contributions based on indiscriminate feeding, in the budget calculations. Moreover, Aller and Yingst (1985) reported that bacterial densities are greatly reduced in fecal pellets of *H. filiformis*, as compared with the surrounding sediment, which implies efficient use of ingested bacterial carbon. Because the estimated uptake of the investigated carbon sources was insufficient to account for the carbon requirements of *H. filiformis*, Clough and Lopez (1993) and Neira and Höpner (1994) have suggested that DOC might be an important additional carbon source. However, no signs of direct ^{13}C -glucose uptake were evident in our experiment (Fig. 1D). In fact, *H. filiformis* was sampled very frequently ($n = 58$), and these observations could be accurately fitted with bacterial $\Delta\delta^{13}\text{C}$ as a forcing function (Fig. 1D). Therefore, we conclude that DOC is an unimportant carbon source for *H. filiformis*.

Another way to elucidate carbon sources utilized by organisms is to examine their fatty-acid composition, in which specific biomarker fatty acids of different sources such as algae, bacteria, or vascular plants can be traced (e.g., Meziane et al. 1997). Bacteria-specific fatty acids have consistently been found in fatty acids of macrobenthos from mangroves (Meziane and Tsuchiya 2000; Bachok et al. 2003) and intertidal sediments (Meziane et al. 1997) and comprise roughly 5% to 15% of the total macrobenthic fatty acids. Similarly, bacteria-specific fatty acids were encountered in all foraminifera at our study site (Moodley et al. unpubl. data). However, conversion of specific fatty acids to a contribution of bacterial carbon in diets is not straightforward. Conversion factors are needed to upscale specific fatty acids to total carbon contribution, assimilation efficiencies may differ among fatty acids, and assimilated fatty acids can be metabolized or deposited as storage fats by the consumer (Iverson et al. 2004). Therefore, a direct comparison with our data is cumbersome, but the presence of bacteria-specific fatty acids in benthic fauna evidently confirms transfer of organic compounds from bacteria to benthic fauna.

The authors are not aware of any study that has evaluated the possible role of feeding/living depth on the contribution of bacteria to faunal diets. In this study, nematodes did not show important differences with regard to living depth (0.08 ± 0.02 , 0.08 ± 0.02 , and 0.06 ± 0.04 for 0–2, 2–5, and 5–10 cm, respectively). The contribution of bacterial carbon for hard-shelled foraminifera was similar for the upper two sediment layers (0.13 ± 0.03 and 0.15 ± 0.05) but was lower for the deepest sediment layer (0.03 ± 0.02). Also no large differences were seen in the contribution of bacterial carbon among macrobenthic feeding modes (Table 1). Although the subsurface feeders *Heteromastus filiformis* (0.21) and *Arenicola marina* (0.23) had a somewhat higher contribution of bacterial carbon, as compared with surface feeders (0.10 to 0.15 on average),

whether these small differences are ecologically relevant remains to be seen. In conclusion, no clear differences were found in the contribution of bacterial carbon in carbon requirements of intertidal metazoan meiobenthos or macrobenthos with respect to sediment depth.

In a companion paper on the fate of bacterial production (van Oevelen et al. 2006), we have shown that benthic faunal grazed 27% of bacterial production ($20 \text{ mmol C m}^{-2} \text{ d}^{-1}$), and this grazing sustained only 18% of the faunal carbon demand ($108 \text{ mmol C m}^{-2} \text{ d}^{-1}$). Consistent with this quantitative ^{13}C mass-balance approach, our study also revealed a limited dependence on bacterial carbon by benthic fauna. The marginal contribution of bacterial carbon to total carbon requirements was surprisingly general among the intertidal meiobenthos and macrobenthos (Table 1). This generality hints at a mechanism that prevents a greater exploitation of bacterial carbon. One possible explanation may be dilution of bacterial carbon by mineral particles and bacterial attachment to mineral surfaces. As a result, bacterial carbon exploitation may be limited by the processing rate of sediment particles by benthic fauna (Cammen 1980). For our study site, we estimate faunal-processing rate as follows: meiobenthic + macrobenthic biomass was $1,872 \text{ mmol C m}^{-2}$ (Table 1), which translates to a volume of $\sim 300 \text{ cm}^3 \text{ m}^{-2}$ ($0.5 \text{ g C} = 1 \text{ g dry wt}$, $0.15 \text{ g dry wt} = 1 \text{ g wet wt}$, and $1 \text{ g wet wt} = 1 \text{ cm}^3$). With a relative gut volume of 0.3 (Penry and Jumars 1990) and gut residence time of 2 hours (Bock and Miller 1999), 1.1% of the upper 10 cm of the sediment passes through a digestive tract on a daily basis. Further, if a homogeneous mixture of bacteria and sediment is assumed, indiscriminate feeding by benthos results in a grazing rate of 0.011 d^{-1} of the bacterial stock. With the average bacteria biomass of $781 \text{ mmol C m}^{-2}$, the expected grazing rate from indiscriminate feeding is $8.4 \text{ mmol C m}^{-2} \text{ d}^{-1}$. Assuming maintenance carbon requirements of $19 \text{ mmol C m}^{-2} \text{ d}^{-1}$ (0.01 of faunal biomass d^{-1} [e.g., Nielsen et al. 1995]), the carbon flux of $8.4 \text{ mmol C m}^{-2} \text{ d}^{-1}$ is below maintenance requirements alone and shows that processing rates by indiscriminate-feeding fauna may indeed limit a greater exploitation of bacterial carbon. In a companion paper, bacterial mortality was shown to be the main fate of bacterial-carbon production, and only part of the production was grazed (van Oevelen et al. 2006).

However, benthic fauna do not ingest sediment particles indiscriminately (Lopez and Levinton 1987). By use of a quantitative model, van Oevelen et al. (2006) derived a faunal-grazing rate for bacteria of $20 \text{ mmol C m}^{-2} \text{ d}^{-1}$, which is about 2.4 times the expectation of indiscriminate feeding ($8.4 \text{ mmol C m}^{-2} \text{ d}^{-1}$). This finding is an indication of selective ingestion of bacterial carbon by benthic fauna. Despite this selective ingestion, bacterial carbon contributed marginally to total carbon requirements (0.10–0.15 [Table 1]). This observation leads us to speculate that fauna select labile organic matter from which they largely derive their carbon requirements. The apparent selection for bacterial carbon can then be explained by covariation of bacterial carbon and labile organic matter, with higher bacterial abundances on more labile organic

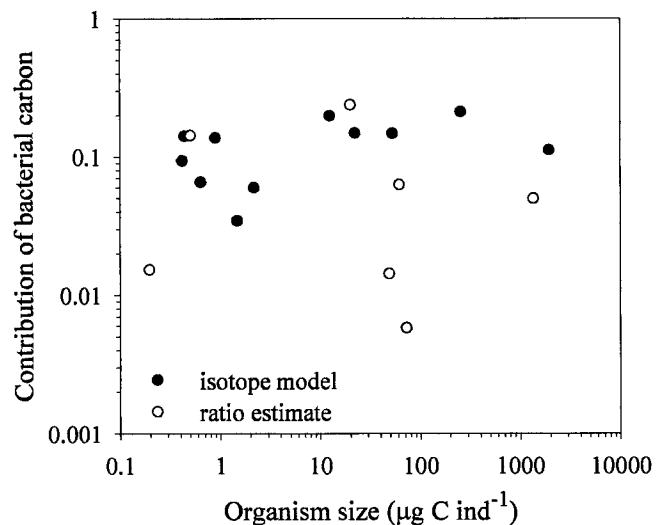


Fig. 3. Relative contribution of bacterial carbon to total carbon requirements as a function of body size.

matter. This covariation has been reported in the literature, although the causality was ambiguous, with lability expressed as chlorophyll *a* concentration (Danovaro et al. 1994; Van Duyl and Kop 1994). This covariation might also explain the observed higher bacterial abundances in ingested sediments, as compared with surrounding sediments, for the deposit feeders *Abarenicola pacifica* (Plante and Jumars 1993), *Arenicola marina* (Andresen and Kristensen 2002), and *Heteromastus filiformis* (Neira and Höpner 1994; Wild et al. 2005).

Animal-foraging theory predicts that feeding niches are defined by body size: small organisms rely on small patches of labile organic matter, whereas larger organisms rely on larger patches of lower lability (Jumars et al. 1990). On the basis of this theory and the covariation of bacteria and labile organic matter, we expect a decreased contribution of bacterial carbon to total carbon requirements with increased body size, on the one hand because of a lower contribution of bacterial carbon from direct grazing on nutritious bacteria by larger organisms and on the other hand because of the covariation between bacteria and lability of organic matter. This expected relation was not evident in our data (Fig. 3), and apparently, intertidal benthic fauna, irrespective of size, depend only for a small fraction on bacterial carbon.

Comparison with other resources and ecosystems—Apart from being a carbon source, bacteria may be a source of nitrogen or essential compounds, such as certain proteins or fatty acids (Lopez and Levinton 1987), which is illustrated by the observation that some foraminifera only reproduced when bacteria were present as a food source (Muller and Lee 1969). Mayer et al. (1995) used an enzyme assay to estimate the bioavailable pool of amino acids in sediments. They suggested that bacterial outer coatings may contribute to this pool and, therefore, be a protein source for deposit feeders. By use of this method, Dauwe et al. (1999) found that a relatively large fraction of bacterial proteins is susceptible to enzymatic attack, as compared

with proteins from sediment samples, which again points to a potential role of bacteria as a protein source. In a companion paper, in which the fate of ^{13}C -peptidoglycan is discussed, a decrease in the ratio of ^{13}C -D-alanine over ^{13}C -L-alanine was attributed to transfer of amino acids from bacteria to benthic fauna (Veuger et al. 2006). Also, the presence of bacteria-specific lipids (i.e., those that cannot be synthesized by fauna) in macrobenthos is an example of transfer of specific bacterial compounds to benthic fauna (Meziane et al. 1997; Meziane and Tsuchiya 2000). Although bacteria may potentially supply limiting or essential compounds to benthic fauna, experimental evidence for the importance of bacterially derived essential compounds for deposit feeders is currently lacking. A combination of isotope addition and compound-specific stable isotope analysis of lipids and amino acids in animals may be used to address this issue. Compounds that are predominantly derived from bacteria are expected to have an elevated $\Delta\delta^{13}\text{C}$, as compared with the $\Delta\delta^{13}\text{C}$ of compounds that are assimilated from other resources. Another logical follow-up experiment would be to apply a dual isotope tracer study with ^{15}N and ^{13}C to determine the importance of bacterial C and N for the benthic food web. ^{15}N enrichment of D-alanine can now be used to determine N incorporation by bacteria (Veuger et al. 2005).

Estuarine intertidal food webs receive labile organic carbon from various sources, (e.g., microphytobenthos and phytodetritus), and the intimate coupling between benthic fauna and these labile carbon sources are important structuring factors for benthic communities (Herman et al. 1999). However, other ecosystems are driven by more refractory carbon inputs, in which case bacterial assimilation may be an indispensable preprocessing step to transform carbon into a more readily digestible form. For example, the carbon input of heterotrophic forest streams is dominated by refractory leaf-litter fall (e.g., high C:N ratio), and the importance of bacterial carbon for higher trophic levels has been well documented for these systems. A ^{13}C -acetate isotope tracer study showed that, although the contribution of bacterial carbon ranged from less than 10% to more than 100%, many stream invertebrates derived more than 50% of their carbon from bacteria (Hall and Meyer 1998). Another example is the soil food web, in which fungi and bacteria are important intermediates in the transfer of detritus to nematodes and microarthropods (Moore et al. 2004, and references therein). Deep-sea sediments are also dominated by relatively refractory organic matter, and a "flash cook" strategy was proposed as a preprocessing step (Jumars et al. 1990): deposit feeders supply the sediment slightly in front of them with oxygen and ammonium and, thereby, stimulate growth of aerobic bacteria, which can then be used as a carbon and nitrogen source. However, direct experimental evidence for the transfer of bacterial carbon to deposit feeders in deep-sea sediments is currently unavailable, and this prediction needs further experimentation. Overall, stream and soil invertebrates appear to derive consistently more carbon from microbes than do intertidal invertebrates, which suggests that microbes are required preprocessors in ecosystems where direct assimilation of the organic detritus

pool is impossible for fauna. In intertidal sediments, where phytodetritus, benthic microalgae, and bacterial carbon are available for assimilation, we conclude that benthic fauna mainly relies on other carbon, such as phytodetritus and benthic microalgae, rather than on bacteria for their carbon.

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