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Differing utilization of glucose and algal particulate organic matter by deep-sea benthic organisms of Sagami Bay, Japan

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ABSTRACT: The fate of particulate and dissolved organic carbon in deep-sea benthic organisms was evaluated by *in situ* ^{13}C-labeling experiments in the central part of Sagami Bay, Japan (water depth: 1453 m). ^{13}C-labeled glucose and *Chlorella* sp. (Chlorophyta) were injected into a series of *in situ* culture cores and incubated for 0 to 9 d. Glucose was chosen as an example of labile dissolved organic matter in the pore water, and *Chlorella* sp. as an example of fresh algal material. Incorporation of both carbon sources by benthic foraminifera and metazoans was determined based on enrichment in ^{13}C of their bulk tissues. Archaeal incorporations were also evaluated by examining ^{13}C-labeled lipid biomarkers. *Chlorella* sp. incorporation by foraminiferal species ranged from 0.0 to 40% of their biomass. Foraminiferal ingestion of algal materials varied markedly among the same species depending on body size or individual differences. All species incorporated glucose to similar extents, ranging from 0.1 to 0.3% of their biomass. Many foraminiferal species incorporated glucose faster than *Chlorella* sp. After 9 d of *in situ* incubation, 26.4, 1.7, 0.1, and 3.8% of added *Chlorella* sp. was detected in the bulk sediment, foraminiferal biomass, examined metazoan biomass, and respired CO_2, respectively. The figures for glucose were 5.3, 0.04, 0.00, and 4.6%, respectively. Labile dissolved organic matter may serve as an accessible food source for benthic organisms and is quickly mineralized on the deep seafloor.

KEY WORDS: Benthic foraminifera · Dissolved organic matter · Particulate organic matter · Benthic ecosystem · Carbon budget · *In situ* tracer experiment

INTRODUCTION

Phytodetritus and its degraded components are thought to be major food sources in deep-sea benthic ecosystems because of their lability (Graf 1987, Pfannkuche 1993), which triggers a rapid response by the benthic community. Rapid responses to phytodetritus input by a number of deep-sea benthic taxonomic groups, including bacteria (Lochte & Turley 1988, Pfannkuche et al. 1999), protozoa (Gooday 1988, Gooday & Turley 1990, Drazen et al. 1998, Kitazato et al. 2000), and megafauna (Tyler & Gage 1984, Campos-Creasey et al. 1994, Witte 1996), have been recorded. Among the benthic organisms, foraminifera are known to play important roles in phytodetritus consumption by ingesting substantial amounts of algae or degraded algal materials (Moodley et al. 2002, Nomaki et al. 2005, 2006, Gooday et al. 2008) and by degrading them (Nomaki et al. 2009), in particular in dysoxic environments (Woulds et al. 2007). Benthic foraminifera also serve as a nutritional source for higher trophic levels, such as some metazoan meio- and macrofauna (Hickman & Lipps 1983, Gudmundsson et al. 2000, Nomaki et al. 2008, Shimanaga et al. 2009). Knowledge of ben-

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thic foraminiferal feeding habits are therefore crucial to understand benthic food webs and resultant biogeochemical cycles on the seafloor.

Among the complex food webs on the seafloor, dissolved organic carbon (DOC) produced *in situ* is assumed to play important roles in addition to phytodetritus. For some benthic organisms, DOC may be an accessible form of nourishment, since the DOC concentration is abundant in pore water relative to overlying water (Burdige 2002). Two agglutinated foraminiferal species from Antarctic shallow water utilize dissolved amino acids directly (DeLaca et al. 1981, DeLaca 1982). The latter 2 studies suggested that foraminifera adapt to highly seasonal oligotrophic environments by utilizing DOC. Dissolved organic matter (DOM) is the largest pool of organic matter in the ocean (Libes 2009). The concentration of DOC in sedimentary pore water is generally higher than that in the overlying seawater by values up to an order of magnitude, although most DOC is presumably refractory (Burdige 2002). This implies that some of the labile molecular components of the DOC could be possible carbon sources for deep-sea foraminiferal species.

The uptake of DOC by a number of benthic taxonomic groups, including bacteria (Cahet et al. 1990, Guilini et al. 2010), benthic foraminifera (DeLaca et al. 1981, DeLaca 1982), marine invertebrates (Shirayama 1992, Baines et al. 2005, de Goeij et al. 2008, Guilini et al. 2010), and whole benthic communities (Sawyer & King 1993, van Oevelen et al. 2006a,b) has been reported. However, most previous studies were limited to shallow-water organisms that can utilize relatively large amounts of organic matter produced on the ocean surface. Since the deep seafloor is generally oligotrophic in comparison with shallow-water areas, responses to labile DOC by deep-sea benthic organisms should differ from those in shallow water.

It is also obvious that archaea, in addition to bacteria, are important components of deep-sea benthic communities, particularly in subsurface sediments (Lipp et al. 2008). However, their activities on the deep-sea floor are still unclear due to difficulties in culturing them in the laboratory.

In the present study, we investigated whether DOC is a suitable carbon source for deep-sea benthic foraminifera and other organisms, including archaea in the bathyal Sagami Bay. The fate of glucose carbon as an example of labile DOC was followed in ^{13}C-tracer experiments. The ^{13}C-tracer experiments were carried out *in situ*, since the metabolism of deep-sea microorganisms is reduced at a pressure of 1 atm (Cahet et al. 1990). ^{13}C-labeled glucose was added to the overlying seawater at the deep seafloor, and subsequent incorporation and mineralization of the label was examined in heterotrophs in the sediments, ranging from archaea to benthic foraminifera to some metazoans. ^{13}C-labeled *Chlorella* sp., as an example of fresh algal materials, was tested in the same way, and the utilization processes of glucose and *Chlorella* sp. were compared. The present study focused on benthic foraminifera, because they are known to consume substantial amounts of organic carbon on the deep-sea floor (Moodley et al. 2002, Gooday et al. 2008). Total uptake rates of ^{13}C-labeled substrates by archaea were not quantified since (1) the archaeal biomass estimation based on the concentration of lipid biomarkers has some variables and (2) the rates of incorporation of ^{13}C to archaeal membrane lipids were highly heterogeneous between glycerol and isoprenoid (Takano et al. 2010), making it difficult to estimate total archaeal uptake rate using a single membrane lipid biomarker.

MATERIALS AND METHODS

***In situ* feeding experiments.** *In situ* feeding experiments were carried out in March 2006 during cruises NT06-04 and NT06-05 of the RV 'Natsushima'. Details of the oceanic settings and benthic processes were reported in a series of papers on Project Sagami (Kanda et al. 2003, Kitazato et al. 2003, Nakatsuka et al. 2003) and on *in situ* experiments (Nomaki et al. 2005, 2006, 2009).

In situ culture cores with a surface sediment area of 52.8 cm^2 (diameter: 8.2 cm, core length: 32 cm) were used in the present study. Details of the *in situ* culture core were described by Nomaki et al. (2009) and Takano et al. (2010). Approximately 600 ml of overlying water was present in the top of the culture cores. Two holes (diameter: 1.5 cm) were left open at the top of the core to facilitate water outflow during core insertion. Every core had two 5 ml syringes that contained ^{13}C-labeled algae, *Chlorella* sp. (2.00 mg C core^{-1}, >98 ^{13}C atom%; Cambridge Isotope) that had been lyophilized and powdered, or uniformly ^{13}C-labeled glucose (6.80 mg C core^{-1}, 98.7 ^{13}C atom%; Cambridge Isotope). The injection part of the syringe was generally located 8 cm above the sediment–water interface (Fig. 1). ^{13}C-labeled *Chlorella* sp. added to the surface sediments corresponded to 379 mg C m^{-2}. The carbon concentration of *Chlorella* sp. corresponded to the daily flux of total organic carbon (TOC) in the central part of Sagami Bay (~200 to 500 mg C m^{-2} d^{-1}; Kitazato et al. 2003, Nakatsuka et al. 2003). The initial carbon concentration of added ^{13}C-labeled glucose in the overlying water (600 ml) of the culture core was calculated to be 944 µM, which is higher than that in the overlying water at this site, but within the range of the DOC concentration in the sediment pore water (~500 to 2000 µM). Since the culture core was not a closed

system, the concentration of ^{13}C-labeled glucose may have decreased during the incubation periods, not only by degradation, but also by diffusion.

In situ culture cores were deployed on the undisturbed seafloor (water depth: 1453 m) by the ROV 'Hyper-Dolphin' (remotely operated vehicle; Fig. 1). Culture cores were maintained some 10s of centimeters away from each other. After positioning the culture cores, ^{13}C-labeled food materials were introduced to the surface sediments. Two *Chlorella* sp. cores were incubated *in situ* for 2 and 9 d (C-2 and C-9, respectively). Three glucose cores were incubated *in situ* for 0, 1, and 9 d (G-0, G-1, and G-9, respectively). Because of limitations in the ROV payload and working time on the seafloor, only a single core was sampled for each substrate and at each time point. The data presented in the present paper need to be interpreted with caution, since it is known that the response of organisms to an artificial food supply varies greatly, especially among macrofauna (e.g. Aberle & Witte 2003). The G-0 core was not examined for the faunal study, but was analyzed for archaeal lipids, bulk sediment, and overlying water.

Additionally, each of the *Chlorella* sp. and glucose cores was incubated *in situ* for 405 d (Takano et al. 2010). Dissolved oxygen concentration in the overlying water of the glucose core incubated for 405 d was 10.4% saturation, which is less than that of ambient seawater (17.7% saturation). This implies that the top part of the core became dysoxic due to less circulation of seawater, but not totally anoxic even after 405 d of *in situ* incubation. The isotopic data on sediment and organisms from cores incubated for 405 d are not reported in the current paper.

Sample processing. Onboard, all recovered culture cores were kept at 4°C prior to core processing (typically within 1 h). Three 20 ml bottles of overlying water samples were gently collected from all cores to determine ^{13}C concentrations of dissolved inorganic carbon (DIC). Soon after collection, the samples were poisoned with $HgCl_2$, sealed with a rubber septum-aluminum cap, and then stored at 4°C prior to further analyses. Sediments were sliced at 1 cm intervals from 0 to 5 cm in depth, followed by the collection of 5 to 7, 7 to 10, and 10 to 15 cm sediment depth samples (Table S1 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf). Subsamples (15 cm^3) of the sediments were used for the analysis of bulk organic matter and archaeal membrane lipid analysis (Takano et al. 2010). These samples were kept frozen at −80°C and then freeze-dried. The remaining sediments at 0 to 5 cm in depth were used for the determination of carbon isotopic compositions of the benthic organisms. They were sieved on a 125 µm mesh with organic carbon (C_{org})-free artificial seawater and then stored at −40°C prior to the isolation of benthic organisms from the sediments.

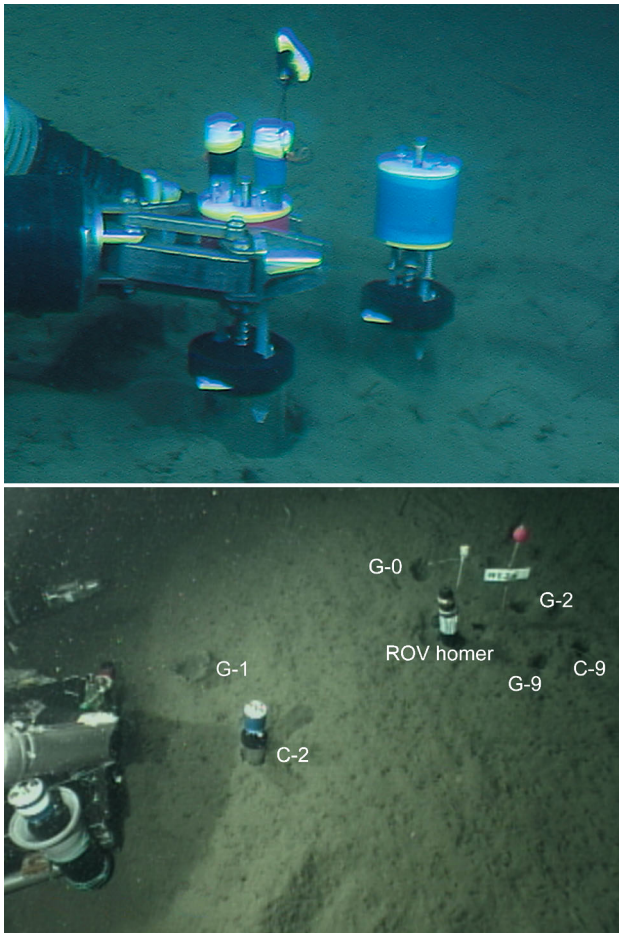

Fig. 1. *In situ* culture cores deployed on the seafloor. Upper panel: deployment of C-9 core (right) and G-9 core (left). Triggers have already been taken off the C-9 core. Lower panel: deployment of *in situ* culture cores and a remotely operated vehicle (ROV) homer. ROV 'Hyper-Dolphin' can be seen on the left

In the laboratory, metazoans and benthic foraminifera, of which the test cavity was filled with cytoplasm, were picked from the sediments under a binocular stereoscopic microscope. Specimens of *Cyclammina cancellata* Brady, *Uvigerina akitaensis* Asano, *Bolivina spissa* Cushman, *Globobulimina affinis* d'Orbigny, and *Chilostomella ovoidea* Reuss were selected for isotopic analyses (Tables S2 & S3 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf). Typically, from 5 to 25 specimens were prepared for each measurement of isotopic composition except for the analyses of *C. cancellata*, for which from 1 to 3 specimens sample^{-1} were prepared. Samples were prepared as replicates if specimen numbers were sufficient. We divided the *U. akitaensis* from the 0 to 2 cm

layer of C-9 into 4 samples: 2 samples consisted of normal, brown cytoplasmic specimens and the other 2 samples consisted of green specimens, which indicated ingestion of the *Chlorella* sp. added to the incubation core (Hemleben & Kitazato 1995). Bathysiphon and mudball (presumably Komokiacea) were also picked and prepared for isotopic analyses.

Some metazoan meiofauna (copepods, meiofaunal size bivalves, and meiofaunal size polychaetes) and macrofauna (cumaceans) were also picked from the sediments. Since sediment samples were examined after freezing and thawing, some fragile taxa such as nematodes were less abundant relative to the *in situ* population (Shimanaga & Shirayama 2000) and were not measured in the present study. A lack of dye would also contribute insufficient collection of metazoan samples from the sediments.

The powdered sediments were weighed and transferred into silver cups that had been prewashed with MeOH and dichloromethane (1:1, v/v; Ogawa et al. 2010). Both foraminifera and metazoans were cleaned with artificial seawater to remove adherent particles. They were transferred into the silver cups and dried at 50°C. All samples were decalcified with 2 N HCl, followed by drying on a hotplate. Dried silver cups containing decalcified samples were sealed into precleaned tin cups prior to isotopic analysis. Although the aqueous acidification procedure sometimes gives altered values of carbon isotopic compositions (up to 0.5‰; Komada et al. 2008), the effect is minor here, since the degrees of ^{13}C-enrichment were enough high.

Archaeal lipid quantification. Archaea is 1 of the 3 domains of life on Earth and is subdivided based on 16S rRNA in 2 major phyla (*Euryarchaeota* and *Crenarchaeota*). Diether lipids (e.g. archaeol) and tetra-ether lipids (e.g. GDGTs; glycerol dialkyl glycerol tetraethers) in the membrane tissue are archaeaspecific biomarkers having isoprenoid moieties (Sinninghe Damsté et al. 2002, Boucher et al. 2004, Koga & Morii 2007, Koga & Nakano 2008). Among these, GDGTs in the marine environment often contain cyclohexane and cyclopentane rings formed by internal cyclisation of the biphytanyl chains, namely GDGT(0), GDGT(1), GDGT(2), GDGT(3), and GDGT(5); GDGT(0) and GDGT(5) are also called caldarchaeol and crenarchaeol, respectively (see Fig. S1 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf) (DeLong et al. 1998, Lipp et al. 2008). By measuring the carbon isotopic compositions of these membrane lipid biomarkers, we evaluated archaeal incorporations of ^{13}C-labeled glucose and *Chlorella* sp. during the experiments.

The method of Takano et al. (2010) for the determination of archaeal lipids, including diether archaeol and putative GDGT, was applied without the copper acetate procedure for the pre-treatment of chlorophyll derivatives. Briefly, after the addition of the internal standard (C_{46} GDGT; Huguet et al. 2006) to freeze-dried sediments (ca. 0.2 g), we extracted archaeal lipids, including archaeol and GDGT, by ultrasonication (20 min) and centrifuged (777 × *g*, 5 min) the extracts 3 times with methanol, 3 times with dichloromethane (DCM):methanol (1:1, v/v), and 3 times with DCM during solid–liquid extraction. All extracts were combined and then dried under a nitrogen flow. Five milliliters of distilled water and 5 ml of *n*-hexane:*n*-propanol (99:1, v/v) were added, and samples were shaken for 1 min for liquid–liquid extraction, a process performed 3 times. The recovered *n*-hexane:*n*-propanol (99:1, v/v) was then dried under a nitrogen flow and redissolved in 1000 µl of *n*-hexane:*n*-propanol (99:1, v/v).

Using high-performance liquid chromatography combined with atmospheric-pressure chemical ionization mass spectrometry (HPLC/APCI-MS, Agilent 1100), the recovered tetraether core lipids were eluted isocratically with 99% *n*-hexane and 1% *n*-propanol for 5 min, followed by a linear gradient to 1.8% *n*-propanol with a flow rate of 0.2 ml min^{-1}. After each analysis, the column was cleaned by back-flushing with *n*-hexane:*n*-propanol (90:10, v/v) at a flow rate of 0.2 ml min^{-1} for 10 min and reconditioned using *n*-hexane:*n*-propanol (99:1, v/v) at a flow rate of 0.2 ml min^{-1} for 10 min. Separation was achieved on a Prevail Cyano column (2.1 × 150 mm, 3 µm; Alltech) fitted with the same packing guard column (4 × 7.5 mm; Alltech). The guard column and main column were maintained at 40°C in a column oven with a preheating system (Polaratherm). Positive-ion spectra were generated by total-ion chromatogram (TIC) scanning of *m/z* (specific mass to charge ratio) 500 to 2000. Although we detected extractable archaeol using cultured *Methanobacterium* sp. (Takano et al. 2009), the amount of archaeol was below the detection limit in the present sediment samples from Sagami Bay.

Determination of organic and inorganic carbon isotope ratios. Carbon isotopic compositions of sediments and benthic organisms along with TOC contents were determined with an isotope ratio monitoring mass spectrometer (Delta plus XP, Thermo-Finnigan) connected to an elemental analyzer (FlashEA1112, CE instruments). The isotope ratios were expressed by δ-notation as $\delta^{13}C$ (‰) = $[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1] \times 1000$. The in-house standard of tyrosine ($\delta^{13}C$: –20.5‰) was used as a working standard. We also determined the carbon isotopic composition of the purified lipid compounds without pretreatment with internal standard quantification using an isotope ratio mass spectro-

meter (Delta plus XP, ThermoFinnigan) coupled with a Flash elemental analyzer (EA1112, ThermoFinnigan) via a Conflo III interface.

^{13}C concentrations of DIC in the overlying water were measured by extracting the CO_2 gas from the seawater samples. In the laboratory, 3 ml of the bottled overlying seawater was mixed with 0.5 ml of pure H_3PO_4 in a vacuum chamber to extract dissolved CO_2 gas in the seawater. The liberated CO_2 with a small amount of H_2O was purified through repeated freezing and thawing. The carbon isotopic ratio of the purified CO_2 was measured using a stable isotope mass spectrometer (IsoPrime, GV Instruments). Standard CO_2 gas ($\delta^{13}C$: −3.64‰) was used as a working standard. The overall precision of $\delta^{13}C$ analyses was ~0.1‰ based on analyses of different aliquots of a seawater sample. When the ^{13}C concentration in a sample was high (i.e. greater than ~100‰), the seawater sample was proportionally (~1:10 to 1:100) mixed with surface seawater that had a known isotopic value (see Table S4 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf). The carbon isotopic composition of the original seawater sample was calculated from the isotope mass balance equation of the measured sample and added surface seawater samples. Those dilution samples were prepared as duplicate or triplicate samples independently to ensure the accuracy of the measurements. Samples diluted at different volume ratios with surface seawater showed similar values (e.g. $\delta^{13}C$ of overlying water DIC from G-9 had values of 4184 and 4273‰ for a sample to surface seawater ratio of 0.00948, and 4185‰ for a ratio of 0.06613), suggesting that the dilution process did not markedly affect the original value. The error between duplicate measurements with high concentrations of ^{13}C showed seemingly high deviation (up to 89‰ in the case of the G-9 core), but overall error for the estimated mineralization rate was only 2.1%, since the labeling was sufficiently great.

Enrichment in ^{13}C was expressed as $\Delta\delta^{13}C$, indicating a relative increase in $\delta^{13}C$ in the sample compared with the background value of natural samples, and was calculated as:

$$\Delta\delta^{13}C\ (‰) = \delta^{13}C_{sample} - \delta^{13}C_{background}$$

$\delta^{13}C_{background}$ values for benthic foraminifera were reported by Nomaki et al. (2008).

Carbon isotopic compositions of foraminiferal samples in the *Chlorella* sp. experiments were also expressed as excess ^{13}C atom% of foraminiferal biomass since some species showed high concentrations of ^{13}C in the samples.

To quantify the inventory of ^{13}C-labeled materials, we calculated the fraction of carbon originating from added food materials in the TOC of the measured sample, f_{sample} (Middelburg et al. 2000, Nomaki et al. 2005, 2006). The amount of incorporated food materials by each benthic organism can be formulated as follows:

$$M = B_{sample} \times f_{sample}$$

where M and B are the amounts of incorporated food material (g C m^{-2}) and biomass of the organism (g C m^2), respectively. Archaeal uptake was not quantified, since the archaeal biomass estimation based on the concentration of lipid biomarkers has some variables, and the incorporation of ^{13}C into the membrane lipid biomarker does not represent the total uptake of archaea.

RESULTS

Mixing of ^{13}C-labeled carbon into sediments

Temporal deliveries of ^{13}C-labeled materials deeper into the sediments were indicated by enriched carbon isotopic compositions of TOC of the bulk sediments (Fig. 2, Table S1 in the supplement). Both ^{13}C-labeled *Chlorella* sp. and glucose were mixed into the sediment to a similar degree.

Peak concentrations of ^{13}C-labeled organic matter were observed in the uppermost 1 cm of the sediments in every time series for the 2 carbon sources. Penetration depths of ^{13}C-labeled organic matter did not differ

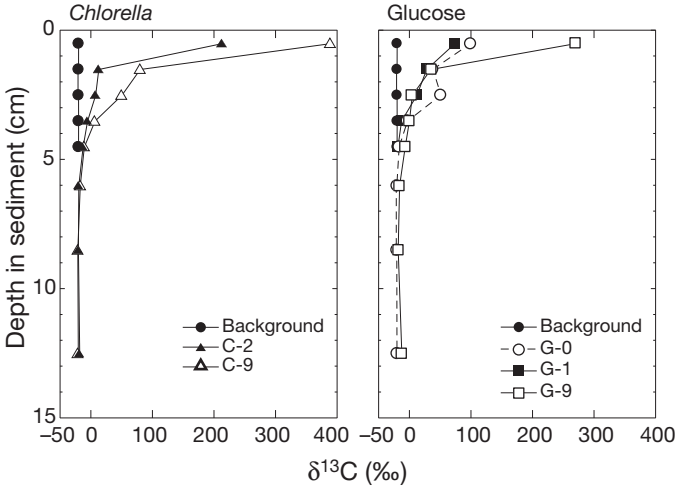

Fig. 2. Vertical profiles of carbon isotopic compositions in experimental cores and a natural background core. Left: profiles of cores to which *Chlorella* sp. was added for 2 or 9 d (C-2 and C-9, respectively); right: profiles of cores to which glucose was added for 0, 1, or 9 d (G-0, G-1, and G-9, respectively). See Table S1 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf for raw data

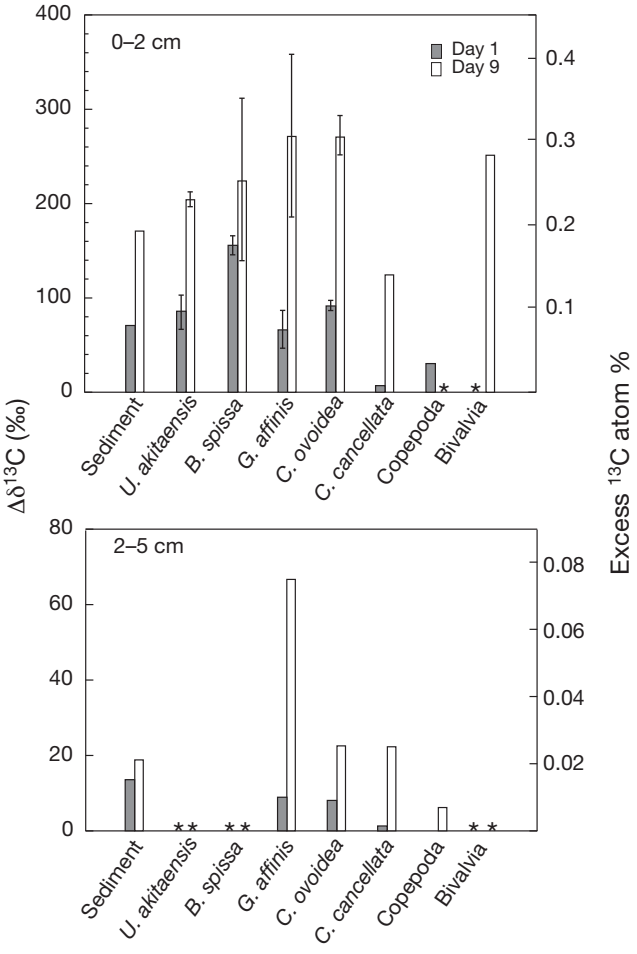

Fig. 3. Enrichment in ^{13}C of sedimentary total organic carbon, benthic foraminifera, and other meio- and macrofauna from cores to which glucose was added. Top: samples from sediments at a depth of 0 to 2 cm; bottom: samples from sediments at a depth of 2 to 5 cm. Error bars: SD (only for cores with replicate samples); *: no data. See Table S2 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf for raw data

markedly as a function of time. In both the C-2 and C-9 cores, ^{13}C-labeled organic carbon was mixed to a depth of 5 cm in the sediments. Significant enrichment was not detected from the sediment layers deeper than 7 cm in the *Chlorella* sp. cores. In the G-0 core, ^{13}C-labeled organic carbon had already reached a depth of 5 cm in the sediments. Although layers deeper than 5 cm were not examined for the G-1 core, the penetration depth of ^{13}C-labeled organic carbon was 4 cm, which was not deeper than that in the G-0 core. ^{13}C-labeled organic carbon penetrated to the deepest part of the sediment layer (10 to 15 cm) in the G-9 core, but the contribution to TOC was minor (–19.0 to –13.3‰ below the 5 cm depth in the sediment).

Incorporation of ^{13}C-labeled glucose by organisms

Foraminifera exhibited modest glucose incorporation, with similar assimilation values among species (Fig. 3, Table S2 in the supplement). On Day 1, we detected ^{13}C originating from glucose in all 5 foraminiferal species in both the shallower and deeper layers. In the shallow layer, the labeling was highest in *Bolivina spissa* with +155.8 ± 10.1‰ (mean ± SD of replicate samples) relative to *Chilostomella ovoidea*, *Uvigerina akitaensis*, and *Globobulimina affinis*, which exhibited $\Delta\delta^{13}C$ of +66.7 to 92.1‰. ^{13}C-labeling of those 4 species was similar to or greater than that in the shallow bulk sediments (+70.9‰). In the deep layer, however, labeling in foraminifera was low for both *G. affinis* (+9.1‰) and *C. ovoidea* (+8.3‰), which was lower than that in the sediment (+13.5‰). *Cyclammina cancellata* showed minor enrichment in ^{13}C in both layers.

On Day 9, 2 deep-infaunal species, *Globobulimina affinis* (+272.1 ± 86.2‰) and *Chilostomella ovoidea* (+272.5 ± 20.8‰), exhibited $\Delta\delta^{13}C$ values higher than

Table 1. Concentrations and carbon isotopic compositions of archaeal core lipid biomarkers in the experimental cores. The concentrations are indicated as average (±SD, n = 3) values. The diether archaeol was not detected in any sample. See 'Materials and methods: Archaeal lipid quantification' and Fig. S1 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf for further explanation of archaeal membrane lipid biomarkers. GDGT: glycerol dialkyl glycerol tetraether

Core	Depth	Tetraether (μg g^{-1} dry wt)								
		Cald-archaeol	$\delta^{13}C_{caldarchaeol}$ (‰)	GDGT1	GDGT2	GDGT3	Cren-archaeol	$\delta^{13}C_{crenarchaeol}$ (‰)	Regio-isomer	Total
G-0	0–1 cm	5.1 ± 1.2	–21.4	1.1 ± 0.3	1.0 ± 0.1	0.2 ± 0.1	5.1 ± 5.6	–21.7	0.7 ± 0.1	13.3 ± 4.0
	2–3 cm	3.5 ± 0.8		0.8 ± 0.2	0.7 ± 0.1	0.2 ± 0.0	7.5 ± 1.8		0.6 ± 0.1	13.3 ± 2.9
G-9	0–1 cm	4.1 ± 0.8	45.7	0.9 ± 0.0	0.7 ± 0.4	0.2 ± 0.0	8.1 ± 0.7	21.9	0.4 ± 0.1	14.5 ± 2.1
	2–3 cm	3.0 ± 1.7		0.6 ± 0.5	0.7 ± 0.3	0.1 ± 0.0	6.0 ± 3.8		0.4 ± 0.1	10.7 ± 6.4
C-9	0–1 cm	3.8 ± 0.2	192.4	1.0 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	7.5 ± 0.1	39.7	0.7 ± 0.4	14.0 ± 0.1
	2–3 cm	3.4 ± 0.6		0.7 ± 0.1	0.8 ± 0.2	0.1 ± 0.1	7.5 ± 1.0		0.7 ± 0.3	13.2 ± 1.7

the shallow-infaunal species even in the shallow layer. *Bolivina spissa*, which exhibited the highest $\Delta\delta^{13}C$ values on Day 1, exhibited $\Delta\delta^{13}C$ values (+225.6 ± 86.1‰) lower than the above 2 deep-infaunal species and similar to that of *Uvigerina akitaensis* (+204.6 ± 7.8‰). *Cyclammina cancellata* was the only species that exhibited $\Delta\delta^{13}C$ values lower than those of the sediment. However, the labeling of *C. cancellata* was much greater on Day 9 than on Day 1.

On Day 9, a Bivalvia specimen from the surface layer showed enrichment in ^{13}C at a concentration similar to those of benthic foraminifera. Copepod specimens from the shallow layer in the G-1 core and from the deep layer in the G-9 core showed slight ^{13}C enrichment in their bodies.

After 9 d of *in situ* incubation, archaeal membrane lipid biomarkers exhibited heavy carbon isotopic compositions, reaching 21.9 and 45.7‰ crenarchaeol and caldarchaeol, respectively (Table 1). ^{13}C enrichment was from +43.6 (crenarchaeol) to +67.1‰ (caldarchaeol) relative to G-0 samples.

Ingestion of ^{13}C-labeled *Chlorella* sp.

Chlorella sp. was ingested by some foraminiferal species within 9 d, but the extent of incorporation differed markedly among species. On Day 2, only *Uvigerina akitaensis* and *Cyclammina cancellata* showed apparent incorporation of ^{13}C-labeled *Chlorella* sp. in both shallow and deep sediment layers (Fig. 4, Table S3 in the supplement). Ingestion by other foraminiferal species, including bathysiphon and mudball (presumably Komokiacea), were negligible on Day 2. By Day 9, *Bolivina spissa* and *Globobulimina affinis* had ingested ^{13}C-labeled *Chlorella* sp. in addition to *U. akitaensis* and *C. cancellata*. The ingestion was apparent in *U. akitaensis* at both depth layers as up to 41% (0 to 2 cm) and 16% (2 to 5 cm) of their C_{org} originated from ^{13}C-labeled *Chlorella* sp. *U. akitaensis* from depths of 0 to 2 cm in the C-9 core showed contrasting ^{13}C-enrichment depending on cytoplasm color (green or brown). The green *U. akitaensis* samples, in which the colour presumably reflects intracellular ^{13}C-labeled *Chlorella* (Hemleben & Kitazato 1995), exhibited 41 ± 1.0% excess ^{13}C atom%, while brown *U. akitaensis* exhibited 4.6 ± 0.08% excess ^{13}C. The other species had lower ^{13}C-labeled *Chlorella* sp. concentrations in their biomass (<2.5%). Large variations in the excess ^{13}C atom% were observed between replicates of *C. cancellata*, which were measured in 1 to 3 specimens for each sample. While the labeling by *U. akitaensis*, *B. spissa*, and *G. affinis* increased from Day 2 to 9, that by *C. cancellata* decreased in the deeper layer. *Chilostomella ovoidea* showed modest ingestion of ^{13}C-labeled *Chlorella* sp. (0.027% excess ^{13}C atom%, i.e. +23.6‰ of $\Delta\delta^{13}C$).

Copepod samples at 2 to 5 cm sediment depth exhibited substantially high ^{13}C atom% values both on Day 2 and 9 (Fig. 4, Table S3 in the supplement). On the other hand, polychaetes and cumaceans were not enriched in ^{13}C on Day 9.

Archaeal membrane lipid biomarkers were enriched in ^{13}C by +61.4 (crenarchaeol) to +213.8‰ (caldarchaeol) on Day 9 relative to G-0 samples (Table 1).

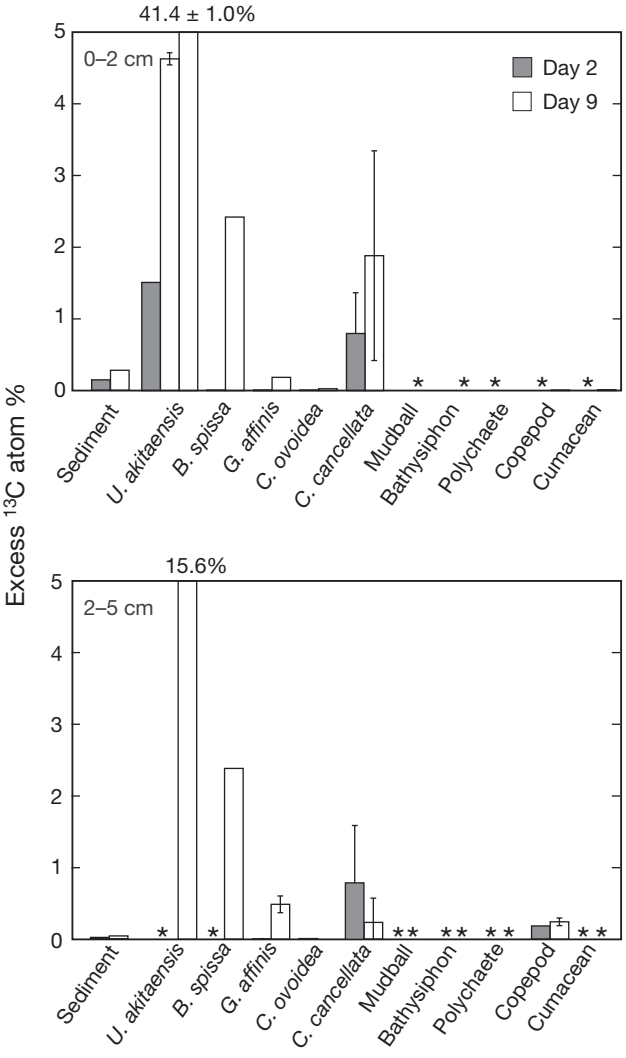

Fig. 4. Enrichment in ^{13}C of sedimentary total organic carbon, benthic foraminifera, and other meio- and macrofauna from cores to which *Chlorella* sp. was added. Top: samples from sediments at a depth of 0 to 2 cm; bottom: samples from sediments at a depth of 2 to 5 cm. Error bars: SD (only for cores with replicate samples); *: no data. Two different Day 9 columns for *Uvigerina akitaensis* in the top panel indicate values for specimens with brown cytoplasm (left column) and green cytoplasm (right column). Values above graphs show the extent of bars with excess ^{13}C atom% >5. See Table S3 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf for raw data

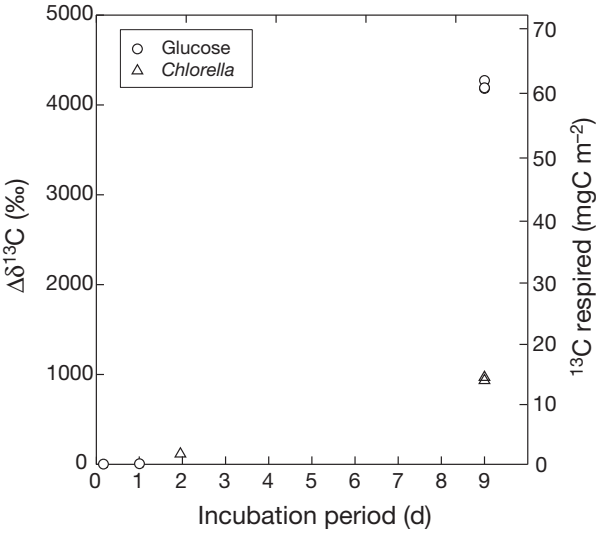

Fig. 5. $\Delta\delta^{13}C$ (‰) of dissolved inorganic carbon extracted from the overlying water collected from the incubation cores. Right axis indicates the amount of respired carbon calculated based on the excess ^{13}C atom%. Note that the 2 axes do not correlate linearly with each other. See Table S4 in the supplement at www.int-res.com/articles/suppl/m431p011_supp.pdf for raw data

Mineralization of ^{13}C-labeled glucose and *Chlorella* sp.

The mineralization rates of the 2 ^{13}C-labeled substrates increased during 0 to 9 d of *in situ* incubation, as reported for the mineralization of ^{13}C-labeled algae addition in onboard experiments (Moodley et al. 2005, Andersson et al. 2008). Benthic mineralization of ^{13}C-labeled substrates was modest within 2 d of incubation in both *Chlorella* sp. and glucose cores (Fig. 5). However, on Day 9, mineralization of label increased drastically with $\delta^{13}C$-DIC values of 958.6 ± 9.5‰ (SD of replicate sample bottles) and 4214.6 ± 41.9‰ in the C-9 and G-9 cores, respectively (Table S4 in the supplement). As a result, the amounts of respired ^{13}C added to the seafloor were 0.11 and 1.9 mg C m^{-2} in the G-1 and C-2 cores, respectively, and drastically increased to 14.4 ± 0.14 in the C-9 and 61.3 ± 0.58 mg C m^{-2} in the G-9 core (Fig. 5).

DISCUSSION

Glucose as an example of DOM in pore water

In the present study, glucose was used as an example of DOM in pore water. Since glucose has a low molecular weight and is easily metabolized by heterotrophs, it may be representative of labile DOC sources in sediments. However, it is known that DOC in the ocean is largely dominated by refractory or semi-labile organic compounds. Labile DOM concentrations represent a small fraction (0 to 6%) of bulk DOM in the open oceans (Carlson 2002). It is thus possible that the glucose used in the present study was not a typical DOC source for benthic fauna.

However, rapid turnover rates of glucose in experimental settings suggest that the fluxes of such labile DOM can be high (Fuhrman & Ferguson 1986, Rich et al. 1996, Keil & Kirchman 1999). Many sources of labile DOM such as bacterial ectoenzyme activities, bacterial cell lysis by viral attacks, and sloppy feeding by grazers support the high flux of labile DOM in the ocean. Because of the rapid cycling of labile DOM, these compounds are present in low concentrations and compose a small fraction of DOM, although the flux rate may potentially be high. Benthic fauna may also utilize such labile DOM. In the sedimentary pore water, the concentration of DOM is generally one order of magnitude higher than that in the overlying water, suggesting high production rates of DOM in sediments.

It was reported that the utilization of glucose differs substantially among different organisms. Glucose uptake contributed from 27 to 35% of net bacterial production in the equatorial Pacific (Rich et al. 1996) and up to 100% in the Arctic (Rich et al. 1997). On the other hand, glucose uptake only contributes <11% of the bacterial production in the Gulf of Mexico and the Antarctic (Skoog et al. 1999, Kirchman et al. 2001). Many bacteria show higher growth rates with amino acids than with glucose and ammonium (Kirchman 1990). In some cases, acetate was a more preferable carbon source for bacteria relative to glucose and amino acids (Guilini et al. 2010). Marine invertebrates prefer fatty acids or algal-derived amino acids to glucose (Baines et al. 2005, de Goeij et al. 2008). It is probable that the incorporation of DOM differs substantially among organisms depending on the types of DOM available. The results of the present study should therefore be considered as specific examples of labile DOC uptake by the benthic community in Sagami Bay.

Incorporation of algae and DOM by foraminifera

The enrichment patterns of benthic foraminifera varied between the 2 ^{13}C-labeled food materials. In the case of ^{13}C-labeled *Chlorella* sp., *Uvigerina akitaensis* ingested food equivalent to up to 40% of their biomass within 9 d, while *Chilostomella ovoidea* showed almost negligible ingestion of *Chlorella* sp. (Fig. 4). On the other hand, all the examined species incorporated similar amounts of glucose (Fig. 3).

We used a single *Chlorella* species as a representative of algal materials although natural phytodetritus contains various materials (Beaulieu & Smith 1998). Furthermore, *Chlorella* sp. has rather refractory cell walls mainly consisting of carbohydrates (Loos & Meindl 1982). However, foraminiferal ingestion patterns of *Chlorella* sp. (Chlorophyta) during long-term incubation were generally similar to those of *Dunaliella* sp. (Chlorophyta) and *Chaetoceros* sp. (diatom) (Fig. 6), suggesting that the observed feeding behaviors of *Chlorella* sp. in the present study were general reactions to algal materials from the water column. Two shallow-infaunal species, *Uvigerina akitaensis* and *Bolivina spissa*, ingested substantial amounts of algae, although the former did so more quickly than the latter (Nomaki et al. 2005, present study). The deep-infaunal species *Chilostomella ovoidea* did not ingest substantial amounts of algae, while another deep-infaunal species, *Globobulimina affinis*, ingested algae slowly, but in measurable amounts. Differences in the foraminiferal response to algal materials in the current study compared with the responses reported in previous studies were: (1) apparent algal ingestion by *Cyclammina cancellata*, which showed negligible ingestion of algae in previous *in situ* feeding studies (Nomaki et al. 2005, 2006) and (2) modest ingestion of *Chlorella* sp. by *B. spissa* and *G. affinis* in the short term (Fig. 6). Previous studies were conducted in April, October, and November, while the present study was conducted in March. The environment on the seafloor in March is similar to that in April, since both are during the spring bloom season (Kitazato et al. 2003), and therefore seasonal changes in feeding behavior may be an unlikely contributing factor to the differences. ^{13}C-labeled algal materials were added to sediments in similar manners in the present (Fig. 2) and previous studies (Nomaki et al. 2005), indicating that access to the food materials did not cause different reactions. Differences in algal species or in algal cell size could cause the different reactions by *C. cancellata*, *B. spissa*, and *G. affinis*. There was no replicate core for each time series, and therefore the different reactions could also be caused by heterogeneous distribution of foraminifera or reactions of individuals. Highly variable ingestion between individuals could also explain the apparent ingestion of *Chlorella* sp. by *C. cancellata* in the present study. It should be noted that the isotopic compositions of *C. cancellata* were typically measured with 1 specimen from each sample in our study (Tables S2 & S3 in the supplement), while previous studies measured it in up to 10 specimens (Nomaki et al. 2005). Data on *C. cancellata* in the present study therefore reflect strong individual variations, whereas data on other species reflect the average incorporation by the species.

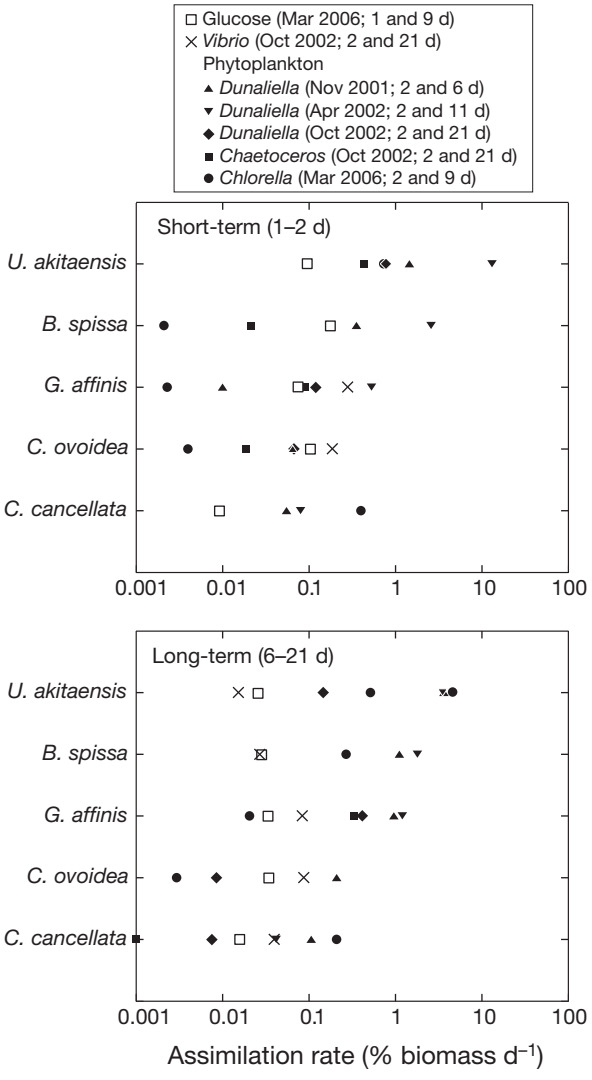

Fig. 6. Incorporation rates of ^{13}C-labeled organic matter by 5 foraminiferal species measured during *in situ* feeding experiments (Nomaki et al. 2005, 2006, present study). Data from the uppermost 2 cm of sediment were weight-averaged and then normalized by dividing by the number of incubation days. The upper and lower graphs are based on short-term (1 or 2 d) and long-term (6 to 21 d) incubation data, respectively

Temporal labeling patterns differed between the glucose and *Chlorella* sp. experiments. Glucose was incorporated quickly by all species, while *Chlorella* sp. was incorporated after a relatively long time by certain species. The incorporation of *Chlorella* sp. increased dramatically with incubation time, while that of glucose increased slightly with time (Figs. 3 & 4). The excess ^{13}C in *Uvigerina akitaensis*, *Globobulimina affinis*, and *Bolivina spissa* in the C-9 core was 15-fold, 41-fold, and 580-fold higher, respectively, than in the C-2 core, whereas these species exhibited a 2.4-fold, 4.1-fold, and 1.4-fold higher excess of ^{13}C in the G-9 rela-

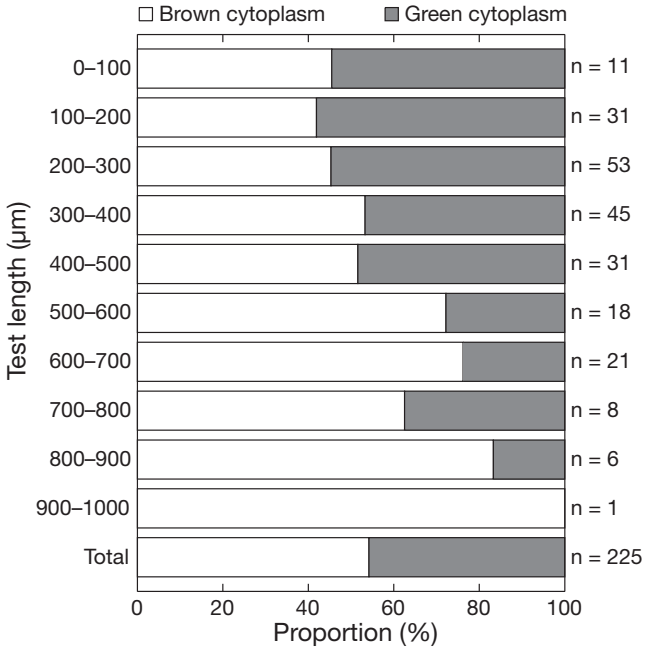

Fig. 7. *Uvigerina akitaensis*. Ratios of specimens with green or brown cytoplasm among different test lengths of *U. akitaensis*. Numbers of individuals in each size range are indicated on the right

tive to the G-1 core. The rapid incorporation of ^{13}C-labeled glucose may reflect absorption through the cell surface (DeLaca et al. 1981). Algal materials were gathered by pseudopodial activity and then incorporated into food vacuoles, resulting in delayed incorporation relative to direct absorption of DOM. Incorporated glucose likely also was respired more quickly than *Chlorella* sp. (Fig. 5), resulting in the lack of assimilation on a longer time scale as seen in the *Chlorella* sp. cores. Phytodetritus may serve as episodic nourishment for some species, while DOC or bacterial carbon serves as a more constant energy source for many species (Fig. 6).

Size-dependent ingestion of *Chlorella* sp. by *Uvigerina akitaensis*

Uvigerina akitaensis from 0 to 2 cm sediment depth in the C-9 core showed contrasting ^{13}C-enrichment depending on cytoplasm color (green or brown). While brown cytoplasm is the typical cytoplasm color for *U. akitaensis*, the green cytoplasm color reflects substantial amounts of *Chlorella*-chloroplast in foraminiferal cells. The green *U. akitaensis* samples exhibited a 41 ± 1.0% excess ^{13}C atom%, while brown *U. akitaensis* exhibited a 4.6 ± 0.08% excess ^{13}C atom%. Although 4.6% represents substantial incorporation of *Chlorella* sp., it is nearly 10-fold lower than that of green specimens collected from the same layer and after the same incubation period. The proportion of green specimens to total specimens was almost equal between the depths of 0 to 2 cm (green: 103 individuals, brown: 122 individuals = 45.8%) and 2 to 5 cm (9:13 = 41%) in the sediments, where the concentrations of ^{13}C-labeled *Chlorella* sp. differed (75.0 and 24.1 mg C m^{-2} of ^{13}C-labeled *Chlorella* sp. or its degraded material in the 0 to 2 and 2 to 5 cm layers, respectively; Fig. 2). This indicates that the modest incorporation of ^{13}C-labeled *Chlorella* sp. by brown specimens was not caused by a lack of access to food material and further suggests that the ingestion of algae differ between individuals even in the same species.

Green specimens comprised 50 to 60% of all specimens <400 µm, but 20 to 40% of those >600 µm (Fig. 7). Lee et al. (1966) observed that smaller (~150 to 200 µm) specimens of *Allogromia laticollaris* ingested more food than larger (350 to 400 µm) ones. Similar size-dependent ingestion of bacteria was observed for the same species, although the numbers of samples were limited (Langezaal et al. 2005). This suggests that larger specimens tend to have a slower turnover rate than smaller ones. Highly variable incorporation between individuals was also observed in *Cyclammina cancellata* in all of our *Chlorella* sp. samples, in which 1 to 3 specimens were examined for each sample (Table S3 in the supplement). Differing responses to algal materials among the same macrofaunal species were also reported at an abyssal Pacific site (Sweetman & Witte 2008). Size-dependent feeding preferences and/or C_{org} turnover may also be an important factor for understanding carbon cycling on the seafloor and the chemistry of the foraminifera.

Enrichment in ^{13}C via microbial processes

The *in situ* experiments were carried out for 9 d. The 9 d interval may be sufficient to induce indirect ^{13}C enrichment of foraminifera or metazoans, i.e. the ingestion of ^{13}C-labeled microbes that had incorporated ^{13}C-glucose to build up their biomass. Guilini et al. (2010) reported that ^{13}C-labeled glucose incorporation into bacterial membrane lipids in the sediments collected from 1280 m water depth took place 1 d after glucose injection and reached a maximum after 2 to 4 d. The data on ^{13}C-enrichment should therefore be considered specifically within this time period.

The enrichment in ^{13}C ($\Delta\delta^{13}$C) of archaeal membrane lipid biomarkers were +43.6 and +67.1‰ in the G-9 core (Table 1), implying that some of the enrichment in foraminiferal carbon could be caused by ingestion of microbes that had incorporated ^{13}C-labeled glucose.

Some benthic foraminiferal species are known to utilize bacterial carbon under experimental conditions (Lee et al. 1966, Bernhard & Bowser 1992, Langezaal et al. 2005). However, the contributions of bacterial carbon to the total carbon requirements of foraminifera are generally low based on direct evaluation in labeling experiments (Nomaki et al. 2006, van Oevelen et al. 2006b, Pascal et al. 2008). Those results suggest that most of the ^{13}C enrichment in the present study did not depend on microbial processes. Moreover, foraminiferal enrichment in ^{13}C in the glucose cores was apparent on Day 1, but did not increase markedly thereafter, suggesting that the enrichment was not through indirect incorporation via microbial processes.

Different fates of *Chlorella* sp. and glucose carbon on the deep seafloor

A similar penetration pattern of *Chlorella* sp. and glucose down to 5 cm (Fig. 2) suggests that bioturbation largely exceeded pore water advection and the diffusion process at this depth, allowing comparable delivery of each ^{13}C-labeled material. Below 5 cm, ^{13}C-labeled *Chlorella* sp. or its degraded matter was not found, while ^{13}C-labeled glucose or its subsequent products were found down to 15 cm. This suggests that DOC can reach far deeper than particulate organic carbon, due to pore water advection or diffusion.

The recovered amounts of ^{13}C-label found as respired carbon, in sedimentary TOC, in foraminiferal biomass, and in metazoan biomass during the experimental periods are summarized in Fig. 8. Since there was no replicate core for each time period, the exact values presented here include some uncertainty.

Mixed ^{13}C was mostly found as sedimentary organic carbon, including benthic biomass, in both *Chlorella* sp. and glucose cores (Fig. 8). After 9 d, foraminiferal biomass was also an important sink for *Chlorella* sp. carbon on the seafloor. Benthic foraminifera stored 1.7% of initially added *Chlorella* sp. carbon, which represents 6.4% of the recovered label in the sediments at the end of the experiments. Little glucose carbon was found in foraminifera or metazoan biomass during the experimental period.

There were time lags of a few days between the settling of ^{13}C-labeled substrates and their mineralization by the benthic community. Only 0.11 and 1.9 mg m^{-2} of added ^{13}C were respired in G-1 and C-2 cores, respectively (Fig. 5). Respired carbon then increased dramatically until Day 9. Roughly half of the recovered carbon was found as respired carbon in glucose cores, indicating rapid mineralization of glucose compared with *Chlorella* sp. carbon mineralization (Figs. 5 & 8). The amounts of respired carbon estimated based on $\delta^{13}C$ values of DIC in the overlying water may be somewhat underestimated because part of the respired carbon escaped from the core thorough the holes in the top (see 'Materials and methods: *In situ* feeding experiments').

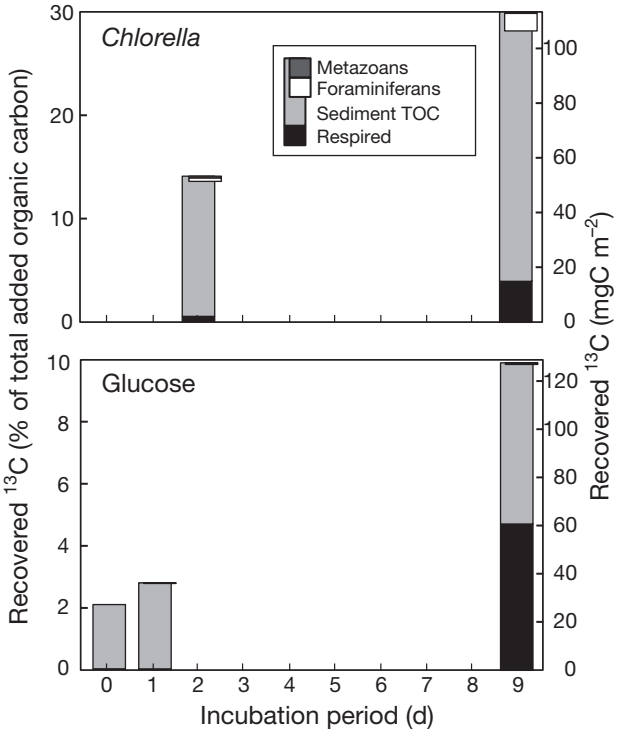

Fig. 8. ^{13}C proportion (of total organic carbon initially added to sediment cores) and mass recovered from dissolved inorganic carbon (respired), metazoans, benthic foraminifera, and sedimentary organic carbon (0 to 15 cm depth), including benthic biomass. Top: cores to which ^{13}C-labeled *Chlorella* sp. was added. Bottom: cores to which ^{13}C-labeled glucose was added. Some of the respired CO_2 may have escaped from the core during incubation. Some metazoan taxa were not included in this calculation. See 'Discussion: Different fates of *Chlorella* sp. and glucose carbon on the deep seafloor' for further explanations

After 9 d of *in situ* incubation, 26.4, 1.7, 0.1, and 3.8% of added *Chlorella* sp. was detected in the bulk sediment, foraminiferal biomass, measured metazoan biomass, and respired CO_2, respectively. The percentages of glucose were 5.3, 0.04, 0.00, and 4.6%, respectively. Nearly half of the recovered ^{13}C in the glucose cores was in the form of respired CO_2, while that of *Chlorella* sp. was found mainly as sedimentary organic matter. Among the benthic fauna, metazoans measured in the present study (polychaetes, cumaceans, bivalves, and copepods) were not responsible for the processing of *Chlorella* sp. and glucose. As some taxa may have been lost during sample processing or isolation (see 'Materials and methods: Sample processing'), total contribution of metazoans may be larger to some extent. Nematodes, which exhibited the second largest

biomass in metazoan meiofauna after copepods at this site (Shimanaga & Shirayama 2000, Nomaki et al. 2005), were not measured in the current study. Although deep-sea nematodes often showed limited uptake of phytoplankton in tracer studies (Nomaki et al. 2005, Ingels et al. 2010, Gontikaki et al. 2011), they could contribute to the DOC processing somewhat (Shirayama 1992) and may have been responsible for some of the glucose processing in our experiments. Some macrobenthos and megabenthos, e.g. Ophiuroida and Spatangoida, also exsist at this site (Nomaki et al. 2008), but they were not sampled in this experiment due to their patchy distribution on the seafloor.

Benthic foraminifera play important roles in phytodetritus processing by assimilating substantial amounts of algal carbon. Their importance is based on both their large biomass (e.g. our study site; Nomaki et al. 2005) and their high capacity of algal ingestion per unit biomass (Moodley et al. 2002, Nomaki et al. 2005, Gooday et al. 2008). Labile DOM may also serve as an accessible food source for certain benthic organisms including foraminifera, but it is then quickly mineralized on the deep seafloor. Although microbial incorporation was not quantified in the present study, the potential abundance of microbes on the deep seafloor and enrichment in archaeal membrane lipids in ^{13}C in the C-9 and G-9 cores (Table 1) suggest a significant contribution by microbes to benthic carbon processes.

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