



Title	Sedimentary membrane lipids recycled by deep-sea benthic archaea
Author(s)	Takano, Yoshinori; Chikaraishi, Yoshito; Ogawa, Nanako O.; Nomaki, Hidetaka; Morono, Yuki; Inagaki, Fumio; Kitazato, Hiroshi; Hinrichs, Kai-Uwe; Ohkouchi, Naohiko
Citation	Nature Geoscience, 3(12), 858-861 https://doi.org/10.1038/ngeo983
Issue Date	2010-11
Doc URL	http://hdl.handle.net/2115/45753
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	article.pdf (本文)



[Instructions for use](#)

Sedimentary membrane lipids recycled by deep-sea benthic archaea

Yoshinori Takano^{1*}, Yoshito Chikaraishi¹, Nanako O. Ogawa¹, Hidetaka Nomaki¹,

Yuki Morono², Fumio Inagaki², Hiroshi Kitazato¹, Kai-Uwe Hinrichs³

5 **& Naohiko Ohkouchi^{1,4,5}**

*¹Institute of Biogeosciences, Japan Agency for Marine-Earth Science and
Technology (JAMSTEC), 2-15 Natsushima, Yokosuka 237-0061, Japan.*

10 *²Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth
Science and Technology (JAMSTEC), Monobe B200, Nangoku, Kochi 783-8502,
Japan.*

*³Department of Geosciences and MARUM Center for Marine Environmental
Sciences, University of Bremen, D-28359, Bremen, Germany*

15 *⁴Department of Environmental Science and Technology, Tokyo Institute of
Technology, 4259 Nagatsuda, Midori-ku, Yokohama, Kanagawa 226-8502, Japan*

*⁵Department of Earth and Planetary Sciences, University of Tokyo,
7-3-1 Hongo, Tokyo 113-0033, Japan*

E-mail: takano@jamstec.go.jp; nohkouchi@jamstec.go.jp

20 Fax: +81-46-867-9775

Since the work of Woese *et al.*¹, Archaea are recognized as one of three domains of life and cosmopolitan microbes in marine environments²⁻⁵. Recent
25 molecular evidence suggests that uncultured heterotrophic archaea relying on relic organic carbon dominate biomass in marine sediment⁶. Archaeal membranes are well characterized, and are comprised of a glycerol backbone and a non-polar isoprenoid chain. However, the ecology of sedimentary archaea remains elusive, because it is difficult to grow them in the laboratory. Here, we trace the fate of
30 ¹³C-labelled glucose added to marine sediments in Sagami Bay, Japan, to determine the *in situ* mechanisms of membrane synthesis. We found that the ¹³C was incorporated into the glycerol backbone of archaeal membranes; ¹³C was apparent after 9 days of incubation, but most pronounced following 405 days. The isoprenoid chain of the membranes remained unlabelled, however. Based on the
35 differential uptake of ¹³C, we suggest that the glycerol unit is synthesized *de novo*, whereas the isoprenoid unit is synthesized from exogenous relic archaeal membranes and detritus, due to the prevalence of these compounds in marine sediments. We therefore suggest that some benthic archaea build their endogenous membranes by recycling sedimentary organic compounds for their growth and
40 maintenance.

Deep-sea sediments harbor a novel and vast biosphere with yet unconstrained importance in the global carbon cycle⁶⁻¹⁴. Carbon isotopic signatures of archaeal polar lipids from sediments dominated by benthic archaeal communities indicate utilization of sedimentary organic carbon^{6,15}. On-shore laboratory studies aimed at understanding

45 microbial processes in deep-sea sediments remain problematic because most indigenous microbes appear viable but non-culturable¹⁶. To further constrain the substrates utilized by benthic archaea, we conducted an *in situ* ¹³C-tracer experiment to provide direct evidence of their metabolism *via* an investigation of membrane lipid biomarkers.

The experiment was performed on the seafloor at the central part of Sagami Bay, 50 Japan (35°00.8'N, 139°21.6'E; 1453 m depth)¹⁷ for 0-405 days (Supplementary Figure 1). Sedimentary processes relevant to carbon burial and benthic activity have been previously documented¹⁸. In our experiment, an aqueous solution of 16.5 mg of ¹³C-labeled glucose (¹³C₆H₁₂O₆) was injected into the headspace of the chamber using dual 5-mL syringes attached to the top (Figure 1). The incubation cores and a reference 55 core were recovered at 0, 9 and 405 days after deployment (hereafter, the culture cores are referred to as G-*i*, with *i* indicating the length of incubation in days).

Already after 9 days, ¹³C-tracer incorporation was unambiguously detected in caldarchaeol ($\delta^{13}\text{C} = +46\text{‰}$) and crenarchaeol (+22‰) from the surface sediment (0–1 cm depth), reflecting the active metabolism and growth of the benthic archaeal 60 community (Figure 2). ¹³C uptake increased after 405 days. Depth profiles of carbon isotopic compositions of caldarchaeol and crenarchaeol are positively correlated ($R^2 < 0.83$; p -value > 0.07) with those of organic matter in the sediment. $\delta^{13}\text{C}_{\text{caldarchaeol}}$ ($< -21\text{‰}$) and $\delta^{13}\text{C}_{\text{crenarchaeol}}$ ($< -22\text{‰}$) in the experimental blank are consistent with values previously observed in benthic¹⁵ and planktonic¹⁹ archaeal communities (Figure 2). 65 ¹³C-tracer uptake process is either due to heterotrophic assimilation of glucose or autotrophic assimilation of ¹³CO₂ after glucose has been oxidized by undefined microbial

community members (cf. DIC, $\delta^{13}\text{C}_{\text{DIC}} > +4200\%$ in G-9 experiment). In the latter case, the signal could result from assimilation of $^{13}\text{CO}_2$ by marine group I (MGI) crenarchaeota. 16S rRNA genes affiliated with the MGI group dominate the corresponding clone library
 70 in the G-9 experiment (Figure 3). However, given previous demonstration of rapid ^{13}C -DIC uptake into the biphytanyl moieties of caldarchaeol and crenarchaeol by closely related planktonic MGI crenarchaeota⁴, this autotrophic scenario appears less likely (see Supplementary Information).

The carbon isotopic compositions of the acyclic, bicyclic and tricyclic archaeal
 75 biphytanes (hereafter referred to as BP[0], BP[2] and BP[3], respectively, see Methods) released from caldarchaeol and crenarchaeol were around -22% in experiment G-405, whereas the bulk GDGT core lipids caldarchaeol ($+69\%$) and crenarchaeol ($+132\%$) were significantly ^{13}C -enriched (Figure 2, Supplementary Tables 1 & 2). This disparity indicates that the ^{13}C tracer is unevenly distributed in the core lipid molecule, *i.e.*,
 80 ^{13}C -tracer is concentrated in the lipid's 2,3-*sn*-glycerol backbone. We can estimate the carbon isotopic composition of 2,3-*sn*-glycerol ($\delta^{13}\text{C}_{2,3\text{-}sn\text{-glycerol}}$) by mass balance (Supplementary Information).

$$86 \times \delta^{13}\text{C}_{\text{caldarchaeol}} = 80 \times \delta^{13}\text{C}_{\text{BP[0]}} + 6 \times \delta^{13}\text{C}_{2,3\text{-}sn\text{-glycerol}} \quad \text{Eq. [1]}$$

$$85 \quad 86 \times \delta^{13}\text{C}_{\text{crenarchaeol}} = 40 \times \delta^{13}\text{C}_{\text{BP[2]}} + 40 \times \delta^{13}\text{C}_{\text{BP[3]}} + 6 \times \delta^{13}\text{C}_{2,3\text{-}sn\text{-glycerol}} \quad \text{Eq. [2]}$$

Accordingly the 2,3-*sn*-glycerol moieties in caldarchaeol and crenarchaeol in the 0–1 cm interval are enriched in ^{13}C by 900‰ and 500‰, respectively, after 9 days, and by

1300‰ and 2200‰ after 405 days (Figure 2). For further confirmation of our observation,
90 the validity of the estimates was confirmed by direct measurement of the carbon isotopic
compositions of 2,3-*sn*-glycerol (Supplementary Information). Then, the carbon isotopic
composition of glycerol moieties derived from the purified caldarchaeol and crenarchaeol
in G-405 samples were up to +1900‰ and +2780‰, respectively. These values are
consistent with ¹³C-mass balance estimations. The large heterogeneity suggests a
95 predominance of the benthic archaeal metabolic pathway toward 2,3-*sn*-glycerol rather
than isoprenoid lipid biosynthesis. The variable differences of $\delta^{13}\text{C}_{2,3\text{-}sn\text{-glycerol}}$ values of
caldarchaeol and crenarchaeol are consistent with changes in community composition and
associated changes of the sources of these two compounds during the course of the
experiment. Structural and biosynthetic studies of archaeal lipids²⁰ suggest the presence
100 of a pathway leading to GDGTs *via* formation of 2,3-*sn*-glycerol and isoprenoid moieties
(Supplementary Information). When synthesizing *sn*-glycerate, Archaea utilize either the
Embden-Meyerhof (EM), the modified EM or the Entner-Doudoroff (ED) pathway²¹. In
contrast, they produce isoprenoids through either the mevalonate (MVA) or the modified
MVA pathway from acetyl-CoA²².

105 16S rRNA and quantitative PCR (qPCR) analysis indicated a community shift in the
composition of the archaeal community and its abundance (10^5 - 10^7 copies g-sed⁻¹) during
the course of the experiment, whereas the relative abundances (%) of tetraether archaeal
lipids were almost constant during 405 days (Figure 3). The initial community consisted
predominantly of *Crenarchaeota* including MGI, the Soil Crenarchaeota Group (SCG),
110 and the Miscellaneous Crenarchaeotal Group (MCG). After 9 days of incubation, the

relative proportion of individual archaeal groups, and especially MGI and MCG, changed substantially. After 405 days, the relative proportions of clones of these two groups resemble again those at initial G-0. Benthic MGI were previously identified in organic-rich (*e.g.*, Peru Margin, ODP Leg 201) and also organic-poor (*e.g.*, Equatorial Pacific, ODP Leg 201) marine sediment¹³. Furthermore, genes of the Methanosarcinales accounted for approximately 20% of the archaeal assemblages after 405 days.

Given the properties of the outer membrane of prokaryotic cells²³ in combination with the extremely energy-limited sedimentary archaeal communities¹¹, the following scenario is consistent with the intramolecular isotopic distribution. Benthic archaea could synthesize caldarchaeol and crenarchaeol via recycling of exogenous precursor compounds, such as biphytane diols resulting from decomposition of decayed archaeal cells, while only 2,3-*sn*-glycerol is synthesized *de novo* process as signaled by exclusive ¹³C-label uptake in this molecular moiety. Price and Sowers²⁴ estimated that the metabolic energy required for growth of microbial cell is 6 orders of magnitude larger than that needed for survival. Therefore, the recycling of fossil molecules could be a crucial strategy for coping with conditions of energy starvation in sedimentary environments and aid in minimizing energy expenditures for growth and maintenance^{12,15}. Transport of these exogenous building blocks may take place *via* permeable channels in the outer membrane²³. Passage of solutes across the outer membrane channel is generally permeable to hydrophobic molecules with molecular weight in excess of 1000 Da²³. Specifically, transport initiated by adsorption of hydrophobic functional groups (*e.g.*,

hydroxyl groups) onto porin proteins are proposed for long-chain fatty acids and other organic molecules across the outer membrane channel ([Supplementary Information](#)).

Relic archaeal tetraether core lipids are among the most abundant molecular types in
135 marine sediments¹⁹; these compounds could potentially serve as substrate for supplying
the building-blocks, although this would require biochemical scission of ether-bonds²⁵, a
process associated with an appreciable investment of energy (<360 kJ mol⁻¹). This
experiment strongly suggests that deep-sea benthic archaea utilize yet unknown
mechanisms to minimize energy expenditures.

140

Methods

Bulk and compound-specific isotope analysis

We extracted archaeal core GDGTs (glycerol dialkyl glycerol tetraethers) from dried
sediments using an improved method²⁶ and purified them by preparative
145 high-performance liquid chromatography combined with atmospheric-pressure chemical
ionization mass spectrometry (HPLC/APCI-MS) without ether-bond cleavage treatment
([Supplementary Figure 2](#)). To verify an analytical bias during HPLC, we carefully
evaluated potential co-elution by fraction collection both before caldarchaeol peak and
after crenarchaeol peak on the chromatogram ([Supplementary Figure 3](#)). This CSIA
150 method was performed with an elemental analyzer coupled to an isotope ratio mass
spectrometer (EA/IRMS). Bulk isotope analysis ($\delta^{13}\text{C}_{\text{TOC}}$) and total organic carbon
(TOC) in the sediment were determined by conventional methods (see [Supplementary
Information](#)).

To analyze the biphytanes of caldarchaeol and crenarchaeol, we performed an ether
155 cleavage treatment²⁷, followed by reduction²⁸. We determined the carbon isotopic
compositions of resulting biphytane and glycerol derivatives using an online gas
chromatograph/combustion/isotope ratio mass spectrometer (GC/C/IRMS), enabling the
(i) calculation of 2,3-*sn*-glycerol ($\delta^{13}\text{C}_{2,3\text{-}sn\text{-}glycerol}$) using the mass balance equation
between caldarchaeol, crenarchaeol and their isoprenoid moieties, (ii) direct measurement
160 of $\delta^{13}\text{C}_{2,3\text{-}sn\text{-}glycerol}$. The validity of this method was verified using another marine
sediment sample prior to application ([Supplementary Information](#)).

2. Phylogenic analysis of 16S rRNA and qPCR for benthic archaeal community

Bulk genomic DNA was extracted from 0.2–0.5 g of sediments using the ISOIL for
165 Beads Beating Kit (Nippon Gene, Tokyo, Japan). Extracted DNA was further purified
using the Montage PCR Kit (Millipore, Billerica, MA), and archaeal 16S rRNA gene
fragments were amplified with Arch21F-1492R primers^{2,29}. The PCR conditions were as
follows: denaturation at 95°C for 60 sec, annealing at 50°C for 60 sec, and extension at
72°C for 120 sec, for 25–30 cycles. A total of 528 clones (more than 160 clones per
170 sample) were selected from the libraries, and the inserted 16S rRNA gene sequences were
determined using a 3130xl Genetic Analyser (Life Technologies, Carlsbad, CA). For
analysing phylogenetic affiliation, the sequences were aligned and grouped using ARB
software (AB561312-AB561824; 513 entries)³⁰. To estimate copy numbers of archaeal 16S
rRNA genes, quantitative PCR (qPCR) was performed with a SYBR[®] Premix
175 DimerEraser[®] (Takara Bio Inc.) by StepOnePlus[™] real-time PCR system⁶ according to the

manufacturer's instructions (Applied Biosystems). Three replicate reactions per sample were performed with the primer set of ARC806F and ARC958R and the thermal condition of 95°C for 30 sec for initial denaturation, up to 50 cycles of 95°C for 5 sec, 55°C for 30 sec, 72°C for 30 sec. The standard curve for archaeal 16S rRNA genes were obtained from
180 genome DNA of *Pyrococcus horikoshii* ($R^2 = 0.995$).

References

- 1 Woese, C. R., Kandler, O. & Wheelis, M. L. Towards a natural system of organisms -
proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl Acad. Sci. USA*.
185 **87**, 4576-4579 (1990).
- 2 DeLong, E. Archaea in coastal marine environments. *Proc. Natl Acad. Sci. USA*. **89**,
5685-5689 (1992).
- 3 Ouverney, C. C. & Fuhrman, J. A. Marine planktonic Archaea take up amino acids. *Appl.*
Environ. Microbiol. **66**, 4829-4833 (2000).
- 190 4 Wuchter, C., Schouten, S., Boschker, H. T. S. & Sinninghe Damste, J. S. Bicarbonate
uptake by marine Crenarchaeota. *FEMS Microbiol. Lett.* **219**, 203-207 (2003).
- 5 Karner, M. B., DeLong, E. F. & Karl D. M. Archaeal dominance in the mesopelagic zone
of the Pacific Ocean. *Nature* **409**, 507-510 (2001).
- 6 Lipp, J. S., Morono, Y., Inagaki, F. & Hinrichs, K.-U. Significant contribution of Archaea
195 to extant biomass in marine subsurface sediments. *Nature* **454**, 991-994 (2008).
- 7 Parkes, R. J., Cragg, B. A. & Wellsbury, P. Recent studies on bacterial populations and
processes in subseafloor sediments: A review. *Hydrogeol. J.* **8**, 11-28 (2000).

Takano *et al.*, *Nature Geoscience*, **3**, 858-861 (2010).

- 8 Rothschild, L. J. & Mancinelli, R. L. Life in extreme environments. *Nature* **409**, 1092-1101 (2001).
- 200 9 D'Hondt, S. *et al.* Distributions of microbial activities in deep subseafloor sediments. *Science* **306**, 2216-2221 (2004).
- 10 Schippers, A. *et al.* Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature* **433**, 861-864 (2005).
- 11 Jorgensen, B. & Boetius, A. Feast and famine-microbial life in the deep-sea bed. *Nature* **205** *Rev. Microbiol.* **5**, 770-781 (2007).
- 12 Valentine, D. L. Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nature Rev. Microbiol.* **5**, 316-323 (2007).
- 13 Inagaki, F. *et al.* Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl Acad. Sci. USA.* **103**, 2815-2820 (2006).
- 210 14 Roussel, E. G. *et al.* Extending the sub-sea-floor biosphere. *Science* **320**, 1046-1046 (2008).
- 15 Biddle, J. F. *et al.* Heterotrophic archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl Acad. Sci. USA.* **103**, 3846-3851 (2006).
- 215 16 Teske, A. & Sorensen, K. B. Uncultured archaea in deep marine subsurface sediments: have we caught them all? *ISME J.* **2**, 3-18 (2008).
- 17 Nomaki, H., Heinz, P., Nakatsuka, T., Shimanaga, M. & Kitazato, H. Species-specific ingestion of organic carbon by deep-sea benthic foraminifera and meiobenthos: In situ tracer experiments. *Limnol. Oceanogr.* **50**, 134-146 (2005).

- 220 18 Kitazato, H. "The project Sagami" - dynamic sedimentary processes of both organic and inorganic materials at continental margins with active tectonic forcing. *Prog. Oceanogr.* **57**, 1-2 (2003).
- 19 Shah, S. R., Mollenhauer, G., Ohkouchi, N., Eglinton, T. I. & Pearson, A. Origins of archaeal tetraether lipids in sediments: insights from radiocarbon analysis. *Geochim. Cosmochim. Acta* **72**, 4577-4594 (2008).
- 225 20 Koga, Y. & Morii, H. Biosynthesis of ether-type polar lipids in archaea and evolutionary considerations. *Microbiol. Mol. Biol. Rev.* **71**, 97-120 (2007).
- 21 Verhees, C. H. *et al.* The unique features of glycolytic pathways in archaea. *Biochem. J.* **375**, 231-246 (2003).
- 230 22 Grochowski, L., Xu, H. & White, R. *Methanocaldococcus jannaschii* uses a modified mevalonate pathway for biosynthesis of isopentenyl diphosphate. *J. Bacteriol.* **188**, 3192-3198 (2006).
- 23 Lodish *et al.*, The outer membrane and cell wall. *In* Molecular Cell Biology, W. H. Freeman & Co Ltd (1995).
- 235 24 Price, P. B. & Sowers, T. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl Acad. Sci. USA.* **101**, 4631-4636 (2004).
- 25 White, G., Russell, N. & Tidswell, E. Bacterial scission of ether bonds. *Microbiol. Rev.* **60**, 216-232 (1996).
- 240 26 Hopmans, E. C., Schouten, S., Pancost, R. D., van der Meer, M. T. J. & Sinninghe Damste, J. S. Analysis of intact tetraether lipids in archaeal cell material and

sediments by high performance liquid chromatography/atmospheric pressure
chemical ionization mass spectrometry. *Rap. Comm. Mass Spectr.* **14**, 585-589
(2000).

245 27 Schouten S., Hoefs M. J. L., Koopmans M. P., Bosch H. J. & Sinninghe Damste, J. S.
Structural characterization, occurrence and fate of archaeal ether-bound acyclic and
cyclic biphytanes and corresponding diols in sediments. *Org. Geochem.* **29**,
1305-1319 (1998).

28 Elvert, M., Suess, E., Greinert, J. & Whiticar, M. J. Archaea mediating anaerobic
250 methane oxidation in deep-sea sediments at cold seeps of the eastern Aleutian
subduction zone. *Org. Geochem.* **31**, 1175-1187 (2000).

29 Lane, D. J. *et al.* Rapid-determination of 16S Ribosomal-RNA sequences for
phylogenetic analyses. *Proc. Natl Acad. Sci. USA.* **82**, 6955-6959 (1985).

30 Ludwig, W. *et al.* ARB: a software environment for sequence data. *Nucl. Acids. Res.* **32**,
255 1363-1371 (2004).

Acknowledgements

The authors express their sincere thanks to three anonymous reviewers for critical and
invaluable reviewing comments. The authors thank T. Terada (Marine work Japan), the
260 shipboard scientists for their experimental and logistic support during cruises
NT06-04,-05,-22 and NT08-02 by R/V *Natsushima* and for providing bathymetric data.
This research was supported in part by a grant from the Japan Society for the Promotion

of Science, Grant-in-Aid for Creative Scientific Research (19GS0211 & 22684030), and the internship program between Japan and Germany (FY2009).

265

Author contributions

Y.T. performed present lipids analysis and wrote the paper; Y.C. & N.O.O. supported on carbon isotope standard reagents and IRMS analysis; H.N. & H.K. ¹³C-substrate set up, *in-situ* deployment of the chamber, core processing during NT06-04, -05, -22 and
270 NT08-02 cruise; Y.M. and F.I. phylogenic molecular analysis of 16S rRNA and qPCR; K-U.H. contributed technical aspects and was involved in study design; Y.T. and N.O. contributed to this study and all authors discussed the results and commented on the manuscript.

275 **Additional information**

Supplementary Information accompanies this paper on www.nature.com/naturegeoscience. Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>. Correspondence and requests for materials should be addressed to Y.T.

280

Figure captions

Figure 1. Distribution of benthic archaeal lipids and their carbon isotopic compositions during *in situ* ¹³C-tracer experiment during 405 days. a, b, Total organic carbon (TOC, wt%) and carbon isotopic composition ($\delta^{13}\text{C}_{\text{TOC}}$, ‰ vs. PDB).

285 **c**, Carbon isotopic compositions of caldarchaeol and crenarchaeol ($\delta^{13}\text{C}_{\text{caldarchaeol}}$
and $\delta^{13}\text{C}_{\text{crenarchaeol}}$) for 0 day (G-0), 9 days (G-9), and 405 days (G-405) experiments.
The experiments using I-K incubation chamber¹⁷ were performed using the ROV
Hyper-Dolphin and the R/V *Natsushima*. Bottom-water properties at the station are
stable throughout the year, with temperatures of $2.3\pm 0.1^\circ\text{C}$ and salinity of
290 $34.5\pm 0.2\text{‰}$ (see also Supplementary Figure 1).

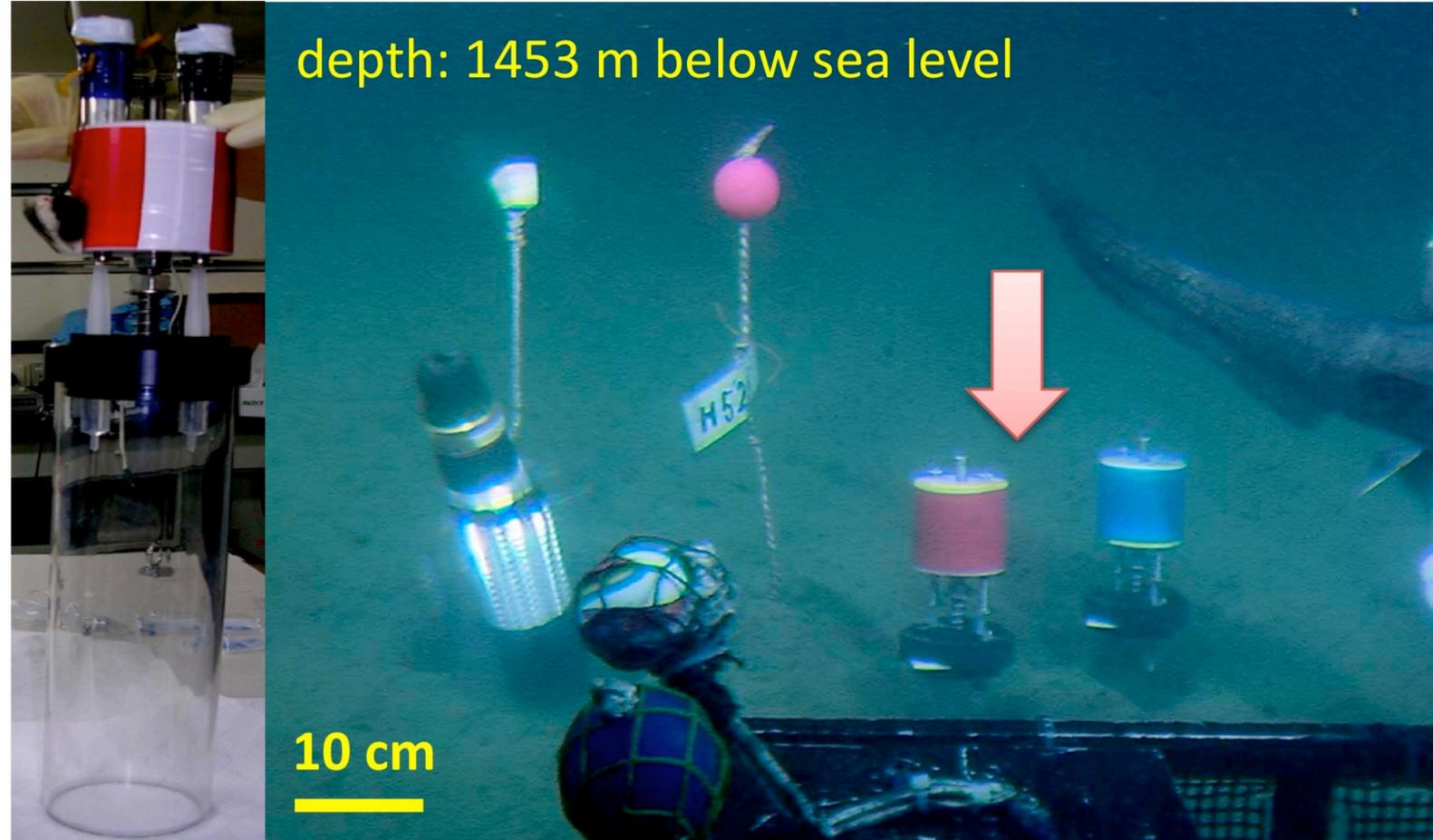
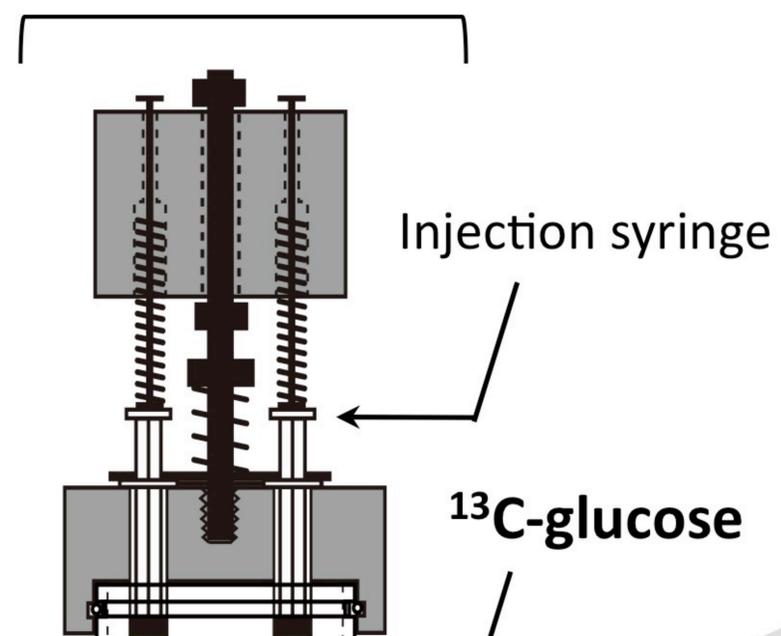
**Figure 2. Results of carbon isotopic compositions of $\delta^{13}\text{C}_{\text{caldarchaeol}}$,
 $\delta^{13}\text{C}_{\text{crenarchaeol}}$, $\delta^{13}\text{C}_{\text{biphytane}}$ and $\delta^{13}\text{C}_{2,3\text{-sn-glycerol}}$ *in situ* ^{13}C -tracer experiments
during 405 days.** The sample analyzed for the time-course profiles was 0–1 cm
depth below the seafloor each. Here, $\delta^{13}\text{C}_{2,3\text{-sn-glycerol}}$ of caldarchaeol and
295 crenarchaeol (*see* molecular structure) were determined as follows: $\delta^{13}\text{C}_{2,3\text{-sn-glycerol}}$
 $= (86 \times \delta^{13}\text{C}_{\text{caldarchaeol}} - 80 \times \delta^{13}\text{C}_{\text{BP}[0]}) / 6$, where $\delta^{13}\text{C}_{\text{caldarchaeol}}$ and $\delta^{13}\text{C}_{\text{BP}[0]}$ were
independently determined by GC/C/IRMS. Furthermore, $\delta^{13}\text{C}_{2,3\text{-sn-glycerol}} = (86 \times$
 $\delta^{13}\text{C}_{\text{crenarchaeol}} - 40 \times \delta^{13}\text{C}_{\text{BP}[2]} - 40 \times \delta^{13}\text{C}_{\text{BP}[3]}) / 6$, where $\delta^{13}\text{C}_{\text{crenarchaeol}}$, $\delta^{13}\text{C}_{\text{BP}[2]}$
and $\delta^{13}\text{C}_{\text{BP}[3]}$ were determined by CSIA. $\Delta\delta^{13}\text{C}_{\text{glycerol-BP}}$ stands for the difference in
300 $\delta^{13}\text{C}$ values between 2,3-*sn*-glycerol and isoprenoid moieties.

**Figure 3. Long-term monitoring for abundances of archaeal lipids and phylogenetic
analysis of the benthic archaeal community. a**, Relative abundances (% , $n=3$) of
GDGTs during 405 days. **b**, Natural assemblage of benthic archaea in the sampling
site (mid-depth 1.5 cm), *in situ* experiment for 9 days and 405 days. Abbreviations;
305 ANME2-d, Anaerobic oxidation of methane 2-d; MBG-D, Marine Benthic Group
D; MEG/TMEG, Miscellaneous Euryarchaeotal Group/Terrestrial Miscellaneous

Euryarchaeotal Group; GSAG, Deep-Sea Archaeal Group; SAGMEG, South African Goldmine Euryarchaeotal Groups; MCG, Miscellaneous Crenarchaeotic Group. Species richness, community diversity, and abundance of the benthic archaea were also monitored during ^{13}C -incubation.

310

In-situ ^{13}C -incubation

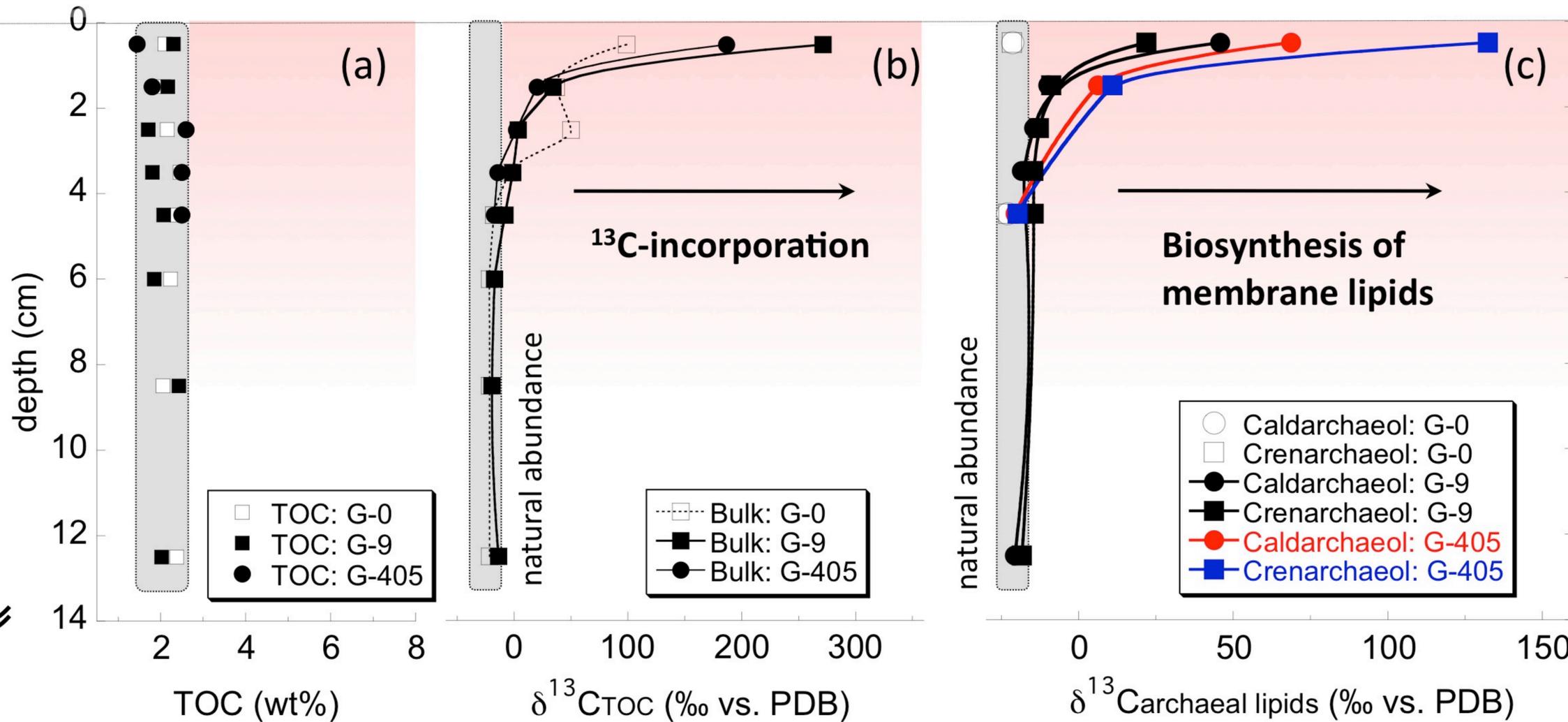


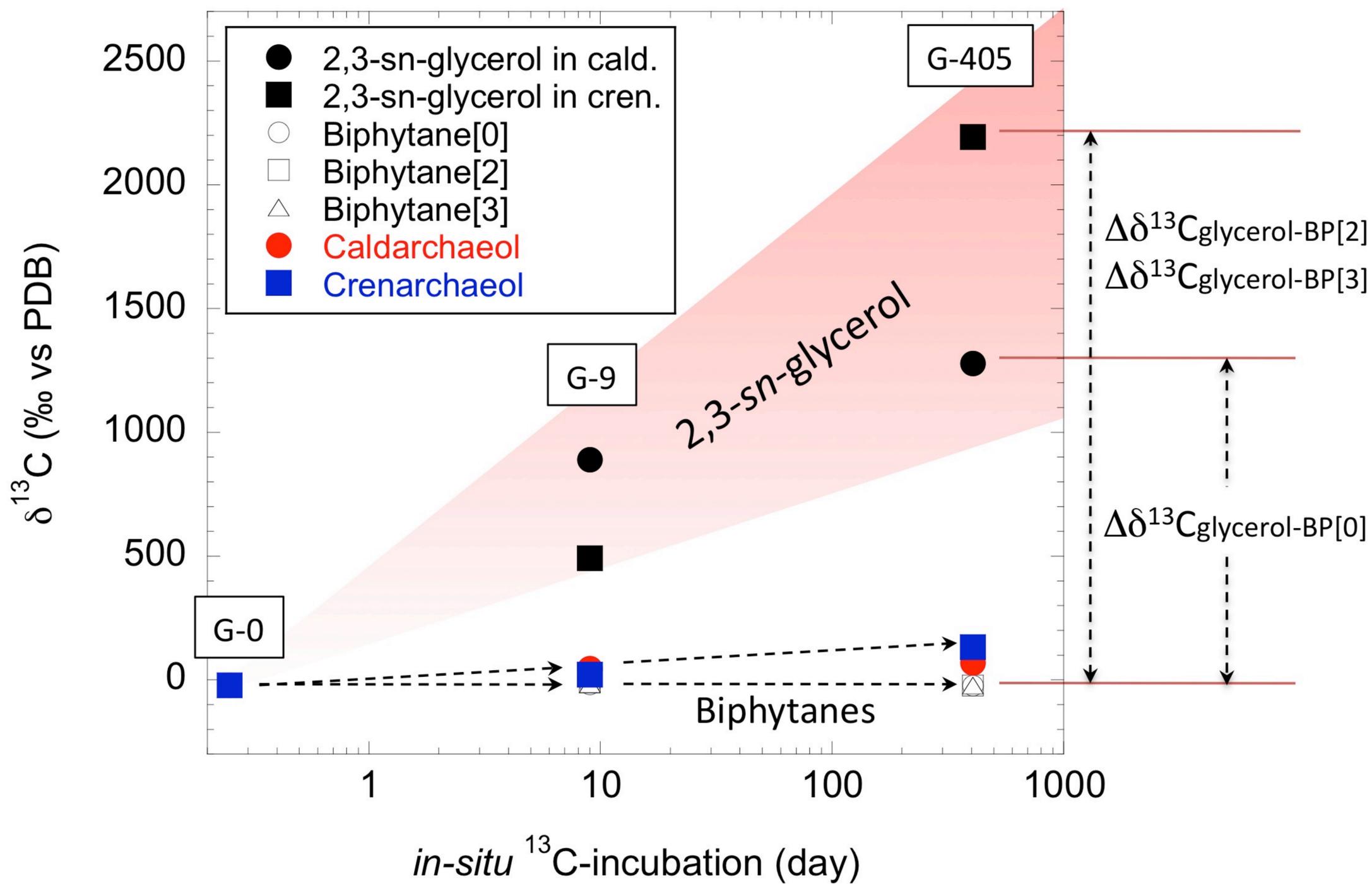
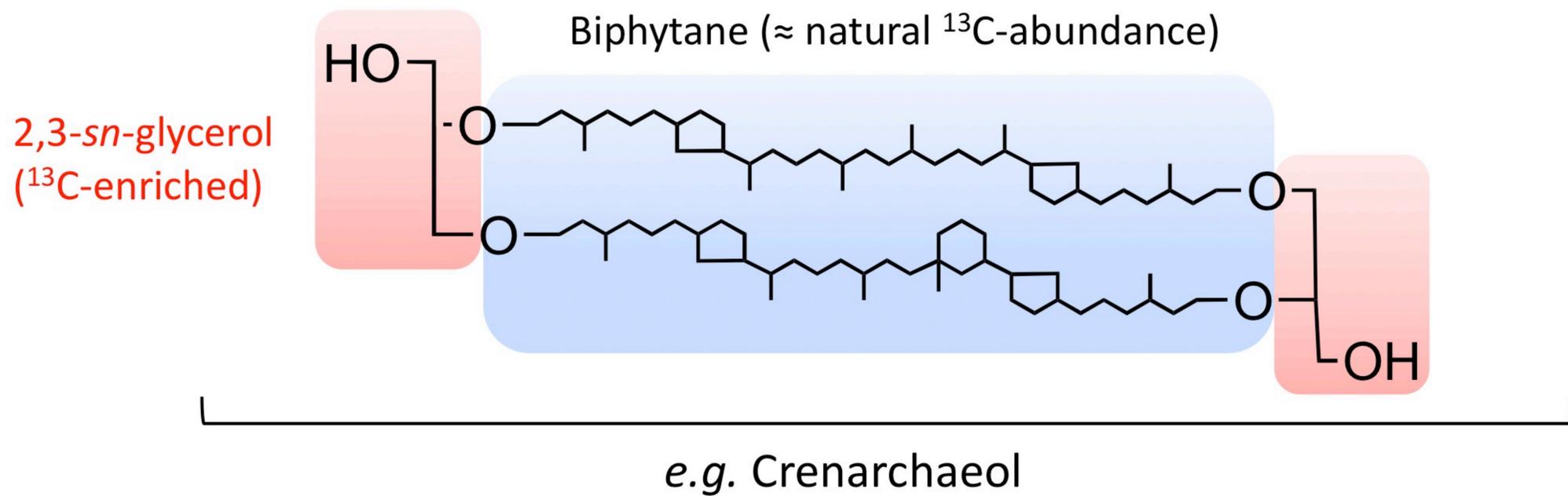
Sedimentary organic carbon

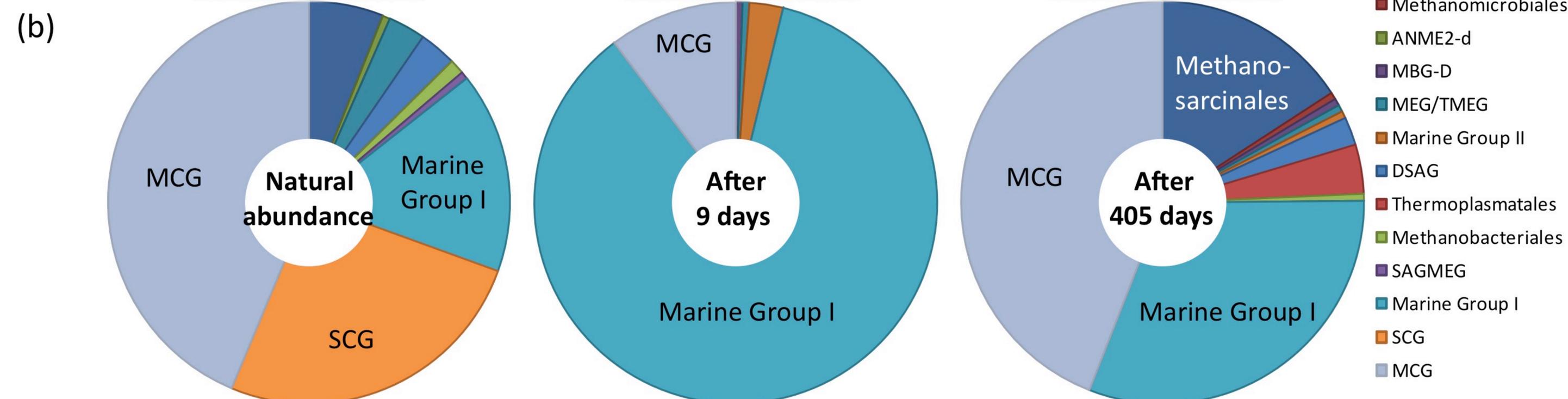
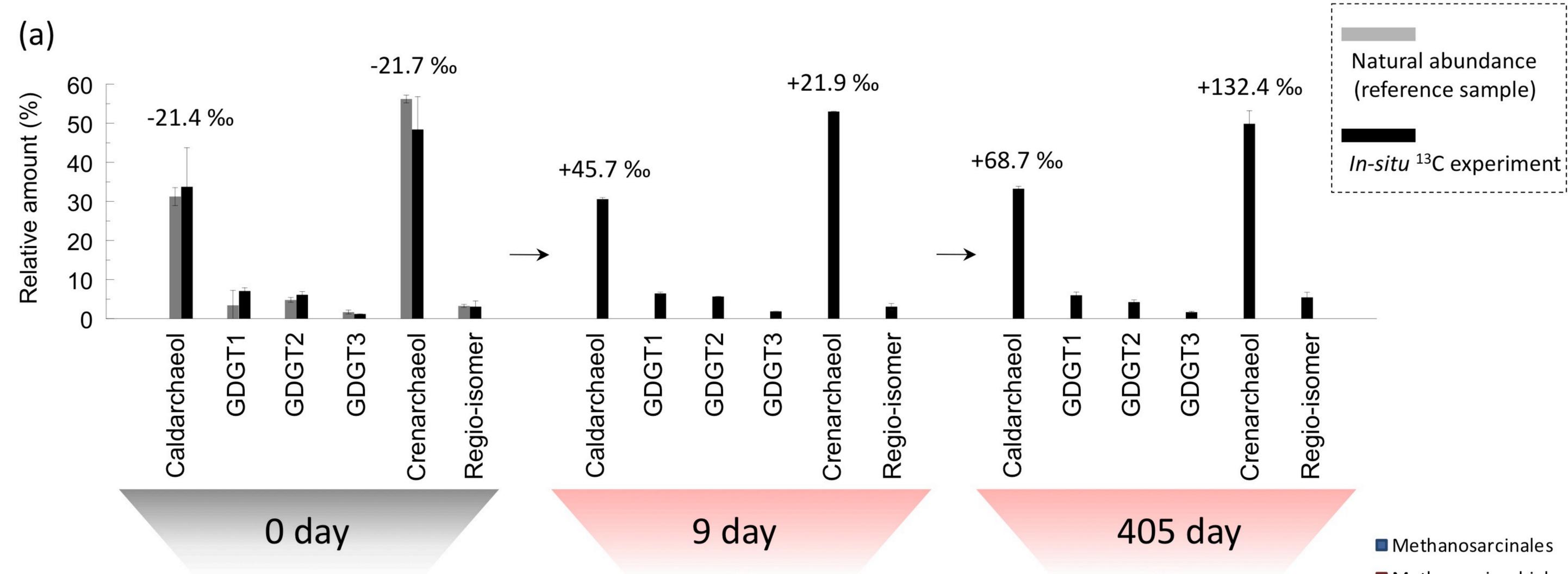
Benthic archaea

water

sediment







Species richness	2647	260	1684	: Chao1 index
Community diversity	4.83	4.22	4.93	: Shannon-Wiener index
Archaeal abundance	1.5×10^6 ($\pm 5.7 \times 10^4$, $n=3$)	8.6×10^7 ($\pm 2.1 \times 10^6$, $n=3$)	1.8×10^6 ($\pm 1.7 \times 10^4$, $n=3$)	: qPCR (copies g-sed ⁻¹)