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Lack of ^{13}C-label incorporation suggests low turnover rates of thaumarchaeal intact polar tetraether lipids in sediments from the Iceland Shelf

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Abstract

Thaumarchaeota are amongst the most abundant microorganisms in aquatic environments, however, their metabolism in marine sediments is still debated. Labeling studies in marine sediments have previously been undertaken, but focused on complex organic carbon substrates which Thaumarchaeota have not yet been shown to take up. In this study, we investigated the activity of Thaumarchaeota in sediments by supplying different ^{13}C-labeled substrates which have previously been shown to be incorporated into archaeal cells in water incubations and/or enrichment cultures. We determined the incorporation of ^{13}C-label from bicarbonate, pyruvate, glucose and amino acids into thaumarchaeal intact polar lipid-glycerol dibiphytanyl glycerol tetraethers (IPL-GDGTs) during 4–6 day incubations of marine sediment cores from three different sites on the Iceland Shelf. Thaumarchaeal intact polar lipids were detected at all stations and concentrations remained constant or decreased slightly upon incubation. No ^{13}C incorporation in any IPL-GDGT was observed at stations 2 (clay-rich sediment) and 3 (organic-rich sediment). In bacterial/eukaryotic IPL-derived fatty acids at station 3, contrastingly, a large uptake of ^{13}C label (up to +80 ‰) was found. ^{13}C was also respired during the experiment as shown by a substantial increase in the ^{13}C content of the dissolved inorganic carbon. In IPL-GDGTs recovered from the sandy sediments at station 1, however, some enrichment in ^{13}C (1–4 ‰) was detected after incubation with bicarbonate and pyruvate. The low incorporation rates suggest a low activity of Thaumarchaeota in marine sediments and/or a low turnover rate of thaumarchaeal IPL-GDGTs due to their low degradation rates. Cell numbers and activity of sedimentary Thaumarchaeota based on IPL-GDGT measurements may thus have previously been overestimated.

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1 Introduction

Thaumarchaea are ubiquitous microorganisms (Hatzenpichler, 2012 and references cited therein) which have recently been discovered to form a kingdom, consisting of one phylum, the Thaumarchaeota (Brochier-Armanet et al., 2008; Spang et al., 2010). They were initially discovered as the Marine Group I Crenarchaeota in the coastal and open ocean (DeLong, 1992; Karner et al., 2001) where they are abundantly present in the epipelagic zone (e.g. Francis et al., 2005; Beman et al., 2008) but also in deep water (Fuhrman and Davis, 1997; Herndl et al., 2005; Agogué et al., 2008) where they can be as abundant as the total bacterial population. Thaumarchaeota have also been detected in marine sediments (Hershberger et al., 1996; Francis et al., 2005).

The metabolism of Thaumarchaeota has long been unclear due to a lack of enrichment cultures. Hoefs et al. (1997) suggested, based on the ^{13}C-enrichment of thaumarchaeal lipids in comparison to algal biomarkers, the possibility of autotrophic assimilation of dissolved inorganic carbon (DIC) using a different pathway than via Rubisco, or, alternatively, the heterotrophic uptake of small organic molecules. Indeed, ^{13}C-labeling studies with bicarbonate showed the incorporation of ^{13}C-labelled DIC into thaumarchaeal membrane lipids and thus their autotrophic metabolism (Wuchter et al., 2003). Unambiguous proof of the growth of Thaumarchaeota on inorganic carbon came with the first enrichment culture of a Thaumarchaeote, *Nitrosopumilus maritimus*, growing chemolithoautotrophically on bicarbonate, and gaining energy by the oxidation of ammonia to nitrite (Könneke et al., 2005). It was suggested that the 3-hydroxypropionate/4-hydroxybutyrate pathway was used by these microorganisms (Berg et al., 2007), which was supported by genetic analyses of *C. symbiosum* (Hallam et al., 2006) and *N. maritimus* (Walker et al., 2010). However, in these two species, also genes involved in heterotrophic metabolisms were detected, which implies their potential for mixotrophy. Furthermore, the uptake of pyruvate into cell material in cultures of a Thaumarchaeote enriched from soil, *Nitrososphaera viennensis*, was demonstrated (Tourna et al., 2011).

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Indeed, there is some environmental evidence for a mixotrophic metabolism of Thaumarchaeota. Ouverney et al. (2000) as well as Herndl et al. (2005) showed, via MICRO-CARD-FISH using ^{3}H-labeled amino acids, that thaumarchaeal cells present in sea water take up amino acids. There is also circumstantial evidence for mixotrophy of Thaumarchaeota, such as the radiocarbon values of archaeal lipids in the deep ocean (Ingalls et al., 2006), thaumarchaeal activity (as quantified by uptake of substrate measured by MAR-FISH) being correlated to the presence of urea (Alonso-Sáez et al., 2012) and the possession of genes for urea transporters (Baker et al., 2012). Mußmann et al. (2011) found thaumarchaeal cell numbers in waste reactors that were too high to be supported by the rates of ammonia oxidation, and a lack of incorporation of ^{13}C-labelled DIC into thaumarchaeal lipids during growth, also suggesting heterotrophy of Thaumarchaeota. Circumstantial evidence, based on the correlation of organic carbon concentration with archaeal biomarker lipids, suggests that Archaea in deep subsurface marine sediments (< 1 m) use organic carbon (Biddle et al., 2006; Lipp et al., 2008; Lipp and Hinrichs, 2009), however, this empirical correlation could also be due to preservation factors affecting biomarkers and total organic carbon in a similar way (Hedges et al., 1999; Schouten et al., 2010).

Cell membranes of Thaumarchaeota consist of glycerol dibiphytanyl glycerol tetraether lipids (GDGT) in the form of intact polar lipids (IPL; Schouten et al., 2008; Pitcher et al., 2011a; Sinninghe Damsté et al., 2012; Fig. 1a). They possess GDGTs which are also present in other Archaea (Koga and Nakano, 2008), such as GDGT-0, −1, −2 and −3 (Fig. 1b), but also a specific lipid named crenarchaeol (Sinninghe Damsté et al., 2002). Stable isotope probing (SIP) experiments targeting the biphytanyl chains contained in crenarchaeol (Fig. 1c), have been undertaken in order to determine the uptake of different substrates. This revealed the incorporation of ^{13}C from bicarbonate into crenarchaeol (Wuchter et al., 2003). Pitcher et al. (2011c) showed that this uptake was dependent on ammonia oxidation in North Sea water, as addition of inhibitors for ammonia oxidation, nitrapyrine (N-serve) or chlorate, resulted in a decrease in incorporation of ^{13}C-labelled DIC. Other SIP experiments carried out with organic

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substrates were less successful: Lin et al. (2013) achieved only a 2 ‰ enrichment in one of the biphytanyl chains of crenarchaeol, but 2 ‰ depletion in the other biphytanyl chain when adding ^{13}C-labeled phytodetrital organic carbon to sediment slurries. Takano et al. (2010) also did not find incorporation of ^{13}C in the biphytanyl chains in an incubation experiment with ^{13}C-labeled glucose in sediment from Sagami Bay, Japan, but remarkably did find labeling of the glycerol moieties of the GDGT molecules. Thus, not much evidence for substantial incorporation of carbon from organic substrates into thaumarchaeal lipids was obtained in SIP experiments performed with sediments. It is possible that the types of substrate used in these studies were not suitable, i.e. not readily taken up by the Thaumarchaeota, and that other organic compounds would result in higher incorporation. Interestingly, none of the previously conducted labeling studies in sediments used ^{13}C-labelled DIC. Bicarbonate had previously unambiguously been shown to be incorporated by pelagic Thaumarchaeota (Wuchter et al., 2003) and by Thaumarchaoeta enriched from sediments (Park et al., 2010) and soils (Jung et al., 2011; Kim et al., 2012).

To shed further light on the metabolism of sedimentary Thaumarchaeota, we performed labeling experiments using ^{13}C-labeled substrates, i.e. bicarbonate, pyruvate, amino acids and glucose in sediment cores from the Iceland shelf. The results are discussed with respect to the uptake of substrates as well as the turnover rates of thaumarchaeal GDGTs.

2 Materials and methods

2.1 Sampling stations

Sediment cores were sampled at three stations, located on the Iceland Shelf, during the "Long Chain Diols" cruise on the R/V *Pelagia* in July 2011 (Fig. 2). Station 1 (63°21′ N 16°38′ W), South of Iceland, was located at 240 m water depth and consisted of dark, sandy sediment. Station 2 (66°18′ N 13°58′ W) is located North-East of Ice-

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land, at 261 m water depth, and the sediment was sandy-clayish. Sediment at Station 3 (67°13′ N 19°07′ W), North of Iceland, at 503 m water depth, and further offshore than the other two stations, consisted of dark, soft, black mud. From each station, multicores were retrieved in polycarbonate core tubes. Ten cores from each station were retrieved in tubes of 10 cm diameter with drilled holes downwards, spaced 1 cm apart, and used for incubation. Cores sampled with tubes of 5 cm diameter were used for oxygen microprofiling, pore-water extraction, and four cores from each station were sliced and stored for density and total organic carbon (TOC) analysis.

2.2 Core characterization

For each station, control cores were sliced into 1 cm thick layers from 0–10 cm core depth. The water content of each sediment was determined by weighing prior to and after freeze-drying. Total organic carbon was determined from freeze-dried sediments following overnight acidification with 2 N HCl, subsequent washes with bidistilled H_2O and water removal by freeze-drying. The decalcified sediments were measured on a Flash EA 1112 Series (Thermo Scientific, Waltham, MA) analyzer coupled via a Conflo II interface to a Finnigan Delta^{plus} mass spectrometer. Standard deviations from three measurements ranged from 0.0 to 0.6 % TOC.

Two cores (5 cm diameter) from each cast were used for oxygen microprofiling with an OX-100 Unisense oxygen microelectrode (Unisense, Aarhus, DK; Revsbech, 1989). A two-point calibration was carried out with bottom water from the same station bubbled with air at 4 °C for at least 5 min (100 % saturation) and a solution of 0.1 M sodium ascorbate in 0.1 N NaOH in Milli-Q Water (0 % saturation) and the electric current measured in pA was converted to mg L^{-1} O_2. The sensor was moved with a micromanipulator one mm at a time.

Pore water was extracted on board from slices of several cores by centrifugation. Five mL from each depth was preserved air-free with added $HgCl_2$ for analysis of the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) at 4 °C, and 3 mL were preserved at −20 °C for analysis of phosphate, ammonia, nitrite and nitrate.

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Dissolved inorganic phosphate, ammonia, nitrite and nitrate were measured on a Traacs 800 Autoanalyzer (Seal Analytical, Fareham, UK). Ortho-phosphate was measured using potassium antimonyltartrate as a catalyst and ascorbic acid as a reducing reagent which resulted in formation of a blue (880 nm) molybdenum phosphate-complex at pH 0.9–1.1 (Murphy and Riley, 1962). Ammonia was measured via formation of the indo-phenol blue-complex (630 nm) with phenol and sodium hypochlorite at pH 10.5 in citrate (Helder and de Vries, 1979). Dissolved inorganic nitrite concentrations were measured after diazotation of nitrite with sulphanilamide and N-(1-naphtyl)-ethylene diammonium dichloride to form a reddish-purple dye measured at 540 nm. Nitrate was first reduced in a copperized Cd-coil using imidazole as a buffer, measured as nitrite and concentrations were calculated by subtraction of nitrite (Grasshoff et al., 1983).

$\delta^{13}C$ of DIC was measured in pore water from all stations, and bottom water samples from the experiments, on a Thermo GasBench II coupled to a Thermo Delta Plus and is reported in the delta notation against theVienna Pee-Dee Belemnite (V-PDB) standard. To this end, 0.5 mL of each sample was injected into gastight exetainers, containing 0.1 mL of 80 % H_3PO_4, that had been flushed for 15 min each with He. Performance was monitored by analysis of an in-house laboratory standard of Na_2CO_3 with a $\delta^{13}C$ of −0.57 ‰ after every 12 measurements and an in-house laboratory standard of $CaCO_3$, with a $\delta^{13}C$ value of −24.2 ‰ at the start and the end of every series. Measured values of these standards were usually not deviating by more than 0.5 ‰ from the calibrated (on NBS-19) values.

2.3 Incubation conditions

Ten cores from each station were used for incubations (Table 2). For this, splitable polycarbonate core tubes with 10 cm diameter were prepared by drilling two rows of 1 mm holes spaced at distances of 1 cm. The two rows were 90° contorted; holes were filled with silicon and covered with plastic non-permeable tape (see setup in Fig. 3). The cores were, after recovery from the multicorer, transferred as quickly as possible to a temperature-controlled room kept at 4 °C, and the bottom water was sampled with a sterile pipette for determination of pH, $\delta^{13}C$ of DIC and nutrients as described above. Four whole cores at each station were incubated with ^{13}C-labelled substrates, i.e. bicarbonate, pyruvate, amino acids and glucose, and one whole core ("background") was injected with MilliQ water only. Four more cores were incubated with solutions containing one of the substrates and a nitrification inhibitor, and one ("background + inhibitor") with MilliQ water and the nitrification inhibitor. The incubation solutions were injected into the seven injection ports from the surface downwards, i.e. the first 8 cm were supplied with the labeled substrate. Injection solutions contained 99 % ^{13}C-labelled substrates as supplied by Cambridge Isotope Laboratories (Andover, MA, USA). The substrates were dissolved in MilliQ-H_2O in concentrations of 3.7 mg mL^{-1} for $NaHCO_3^-$ (Bic), 1.01 mg mL^{-1} for Glucose (Glu), 0.89 mg mL^{-1} for a mix of 16 algal amino acids (AA; 98 % labeled) and 1.24 mg mL^{-1} of sodium pyruvate (Pyr). 200 µL were delivered to each slice by injection through the two 90° contorted injection ports, which corresponded to a total addition of 1.6 g ^{13}C m^{-3} sediment for the bicarbonate, and 1.2 g ^{13}C m^{-3} for the organic compounds. The nitrification inhibitor was Nitrapyrine (N-Serve, Pestanal) at 11.5 µg mL^{-1}, which is known to inhibit archaeal ammonia oxidation (Park et al., 2010; Pitcher et al., 2011c). As Nitrapyrine cannot be dissolved in pure water, 5 % of EtOH was added and the solutions were kept at 30 °C to prevent precipitation of the Nitrapyrine. Nitrapyrine-free incubation solutions were kept at the same conditions.

After injection, the cores were stored in the dark at 4 °C in a water bath – boxes with core holders filled with water- for 62 h (38 h for Station 3), after which the bottom water was sampled for ^{13}DIC and N- and P-nutrients as described above and re-injected with a fresh solution of the substrates in water, with or without nitrapyrine added. During the incubation time, the cores were covered lightly with a rubber stopper, so that the escape of air was possible, and aerated through a sterile plastic pipette, with air pumped by an aquarium pump and filtered through a 0.2 µm filter for sterilization purposes. After

144 h (96 h for Station 3), bottom water samples for ^{13}DIC (dissolved inorganic carbon) and N- and P-nutrients were taken again, and oxygen microprofiles were measured as described above on all cores (except for Station 2, where cores were too short and thus surfaces too low in the core tubes, to allow measurements in more than three cores). Subsequently, the upper 10 cm of the sediment were sliced in 1 cm resolution and each slice mixed thoroughly, split in half and both parts were frozen at −80 °C immediately. The incubations at station 3 were shorter due to time constraints.

2.4 Lipid extraction, separation and measurements

The freeze-dried sediments of 0–1 cm, 1–2 cm and 7–8 cm depth intervals of the incubation and background cores were ground and extracted by a modified Bligh-Dyer extraction method (Pitcher et al., 2009). Briefly, they were extracted ultrasonically three times in a mixture of methanol/dichloromethane (DCM)/phosphate buffer (2 : 1 : 0.8, $v:v:v$), centrifuged and the solvent phases were combined. The solvent ratio was then adjusted to 1 : 1 : 0.9, $v:v:v$, which caused the DCM to separate. Liquid extraction was repeated two more times, the DCM fractions were combined, the solvent was evaporated and bigger particles were removed over cotton wool. An aliquot of the extracts was stored for high performance liquid chromatography/electron spray ionization tandem mass spectrometry (HPLC/ESI-MS^2) analysis, and another aliquot was separated into core lipid (CL)- and intact polar lipid (IPL)-GDGTs by silica column separation with hexane/ethyl acetate (1 : 2, $v:v$) for the CL-fraction and MeOH to elute the IPL-fraction. 0.1 mg C_{46} internal standard (Huguet et al., 2006) were added to the CL-fractions. The IPL-fraction was split in three aliquots: 5 % were used to determine the carry-over of CL-GDGTs into the IPL-fraction and measured directly via HPLC/APCI-MS, 60 % were kept for ether cleavage in order to determine the ^{13}C incorporation into the biphytanes (see below), and 35 % were subjected to acid hydrolysis under reflux for 2 h with 5 % HCl in MeOH, followed by addition of water and extraction (3×) of the aqueous phase at an adjusted pH of 4–5 with DCM. This yielded the IPL-derived GDGTs

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which were measured via HPLC/APCI-MS. The C_{46} internal standard was used to quantify the IPL-derived GDGTs and the carry over, but was added after the aliquot for ^{13}C analysis was taken in order to prevent any possible interference of alkyl chains released from the internal standard molecule.

2.5 $\delta^{13}C$ analysis of biphytanes

Aliquots (60 %) of the IPL-fractions were subjected to chemical treatment in order to cleave GDGTs, releasing the biphytanyl chains (Fig. 1c) according to Schouten et al. (1998). For this, the IPL fraction was refluxed in 57 % HI for 1 h to break the ether bonds and produce alkyliodides and subsequently extracted three times with hexane. The hexane phase was washed with 5 % NaS_2O_7 and twice with water. The alkyliodides were purified over Al_2O_3 with hexane/DCM (9 : 1, *v* : *v*), hydrogenated with H_2/PtO_2 in hexane for 1 h (Kaneko et al., 2011) and eluted over Na_2O_3 with DCM. As the H_2/PtO_2 hydrogenation-procedure has never been tested for stable carbon isotope analysis, we used four CL-fractions of extracts from Station 1 and Station 2, split them in half and hydrogenated one half with the conventional $LiAlH_4$ reduction method described by Schouten et al. (1998), the other half with the method described here and by Kaneko et al. (2011). The conventional method consisted of $LiAlH_4$ in 1,4-dioxane for 1 h under reflux, then the remaining $LiAlH_4$ was reacted with ethyl acetate, bidistilled H_2O was added and the biphytanes were extracted with DCM from the dioxane/H_2O mixture. Additional purification was achieved by elution over an Al_2O_3 column using hexane. The results showed that the $\delta^{13}C$ values of the biphytanes were not significantly different when H_2/PtO_2 treatment and $LiAlH_4$ hydrogenation were compared (Table 1). However, when yields were compared, it was obvious that hydrogenation with H_2/PtO_2 gave better results. This is probably due to the fact that the H_2/PtO_2 treatment requires fewer workup procedures after hydrogenation (only one drying step) than the $LiAlH_4$ treatment, which requires two column separations in addition to a drying step.

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2.6 $\delta^{13}C$ of phospholipid-derived fatty acids

In order to demonstrate that the incubation experiments did result in label uptake by the sedimentary microbial community, we determined the uptake of ^{13}C-label by bacteria and eukaryotes by isotopic analysis of polar lipid-derived fatty acids (PLFA). For this, one third of the extract of the 0–1 cm sediment slice from Station 3, from the cores incubated with bicarbonate, glucose, amino acids and the background core (all without nitrapyrine), were used and analyzed for PLFA according to Guckert et al. (1985). Briefly, the aliquots were separated over a silica column with DCM, acetone and methanol, respectively, with the methanol-fraction containing the PLFA. PLFA fractions were saponified in methanolic KOH (2 N) and, after adjusting to pH 5, the resulting fatty acids were extracted with DCM and methylated with BF_3-MeOH (with a $\delta^{13}C$ of -25.4 ± 0.2 ‰ V-PDB) before measurements by gas chromatography – isotope ratio mass spectrometry (GC-irMS). The $\delta^{13}C$ values for the fatty acids were corrected for the added carbon.

2.7 HPLC-APCI-MS and HPLC-ESI-MS^2

The CL-fractions, hydrolyzed IPL-fractions and the IPL-fractions themselves (to determine the carry-over of CL into the IPL fraction) were analyzed by HPLC-APCI-MS as described previously (Schouten et al., 2007). HPLC-ESI-MS^2 in selected reaction monitoring (SRM) mode, as described by Pitcher et al. (2011b), was used to analyze selected IPL-GDGTs. IPL-GDGTs monitored were monohexose (MH)-crenarchaeol, dihexose (DH)-crenarchaeol and hexose, phosphohexose (HPH)-crenarchaeol. Performance was monitored using a lipid extract of sediment known to contain the monitored GDGTs that was injected every 8 runs. Response areas per mL sediment (mL sed) are reported, as absolute quantification was not possible due to a lack of standards. The values reported are the averages of duplicate injections and their standard deviations.

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2.8 GC-MS and GC-irMS

GC-MS was used to identify the biphytanes and phospholipid-derived fatty acid methyl esters (FAME) using a TRACE GC with a DSQ-MS. The gas chromatograph was equipped with a fused silica capillary column (25 m, 0.32 mm internal diameter) coated with CP Sil-5 (film thickness 0.12 µm). The carrier gas was helium. The compound specific isotope composition of the biphytanes and fatty acids was measured with an Agilent 6800 GC, using the same GC column conditions, coupled to a ThermoFisher Delta V isotope ratio monitoring mass spectrometer. The isotopic values were calculated by integrating the mass 44, 45 and 46 ion currents of the peaks and that of CO_2-spikes produced by admitting CO_2 with a known ^{13}C-content into the mass spectrometer at regular intervals. The performance of the instrument was monitored by daily injections of a standard mixture of a C_{20} and a C_{24} perdeuterated n-alkane. Two replicate analyses (one in the case of the fatty acids) were carried out and the results were averaged in order to obtain the average and standard deviations. The stable carbon isotope compositions are reported in the delta notation against the V-PDB standard. Some values of the biphytanes could not be determined due to low concentrations, mainly in the background cores at station 2. For calculations of incorporation thus, the background values from station 1 were used.

3 Results

3.1 Sediment characteristics

The sediments at stations 1–3 consisted of sand, sandy clay, and dark and soft mud, respectively. Oxygen penetration depth was on average 17 mm at station 1, while oxygen penetrated slightly less deep at station 2 (13 mm on average) and station 3 (15.5 mm on average; Fig. 4). At station 1, organic carbon contents were low with 0.6 % at 0–1 cm depth and 0.3 % at 1–2 cm and 6–7 cm depth. Organic carbon contents were higher at station 2 and amounted to 1.4–1.7 % and were highest at station 3 (2.0–2.5 %). The pH of the bottom water hardly differed between stations and was typically marine, i.e. 8.2 at station 1, 8.0 at station 2, and 7.9 at station 3.

Pore water concentrations of nutrients, as shown in Fig. 4, showed similar trends with depth at the three stations. Inorganic phosphate concentrations were 1 µmol L^{-1} in the bottom water and generally increased with depth up to 7–14 µmol L^{-1}. Ammonia concentrations increased as well with sediment depth, from 0.19 to 1.3 µmol L^{-1} in bottom water to 44–70 µmol L^{-1} at depth. Nitrite concentrations were an order of magnitude higher in the pore water (0.32–0.62 µmol L^{-1}) than in the bottom water (0.055 µmol L^{-1}), and increased with sediment depth. Nitrate concentrations were high in the bottom water, 14 µmol L^{-1}, and generally decreased quickly with depth in the sediment to values between 0.6 and 3 µmol L^{-1}. The δ^{13}C of the DIC showed also similar profiles at the three stations. It decreased from 1 ‰ in the bottom water to −3 ‰ in the pore water. At station 2, δ^{13}C of the DIC could only be measured in the bottom water and the four uppermost pore water samples, but, in these samples, showed the same decrease with depth as observed at station 1, from −1.3 ‰ to −2.9 ‰.

3.2 GDGT-abundance

GDGT-concentrations averaged over all cores from the incubations (10 per station) are shown with standard deviation in order to give an impression of the range of concentrations found (Fig. 5). GDGT-0 and crenarchaeol were present as a core lipid in concentrations of 0.4 to 0.8 µg (mL sed)$^{-1}$, and 0.08 to 0.12 µg (mL sed)$^{-1}$ for the minor isoprenoid GDGTs (i-GDGTs; GDGT-1, −2 and −3). Concentrations were similar at all stations and did not show substantial changes with sediment depth. IPL-derived GDGTs were present in lower concentrations and amounted to 0.09 to 0.3 (GDGT-0), 0.07 to 0.18 (crenarchaeol) µg (mL sed)$^{-1}$. Concentrations were generally lower at station 1 compared to stations 2 and 3.

Direct analysis of individual IPL-crenarchaeol showed that the head groups mainly consisted of monohexose (MH)- and hexose, phosphohexose (HPH)- headgroups, with only a small contribution of dihexose (DH)- head groups (Fig. 5c). The proportion of HPH- to the total peak area of IPL-crenarchaeol was ca. 80–90 % in the surface sediments (0–1 and 1–2 cm) of all stations. At station 1 and 2, this decreased to 10–25 % in the deepest sediment slice analyzed, 6–7 cm. At station 3, HPH-crenarchaeol dominated at all sediment depths (Fig. 4).

3.3 Incubation experiments

Incubations experiments were started directly after core recovery and initial sampling by injection of solutions, which contained ^{13}C-bicarbonate, pyruvate, glucose, amino acids or just water (incubated for comparison and further called the "background core") over the first 7 cm of the cores. The cores were incubated at in situ temperatures (4 °C) for 6 days (4 days for station 3) and subsequently sliced and stored for analysis of ^{13}C-enrichment in lipids. Oxygen penetration depths were measured and bottom water was sampled at the start of the experiment, after 48 h (36 h for Station 3) and at the end of the incubations. At all stations, nutrient concentrations of the bottom water did not change over the 6 days (5 days for station 3) of incubation, except for nitrate, which generally showed a decrease (Table 2). The bottom water pH remained mostly constant, except at station 1, where it changed during the incubation from 8.2 to 8.0 in all incubation, similar to the other sediment cores and perhaps indicating that the initial value of 8.2 might have been due to an error in the measurement. The δ^{13}C of the DIC in the bottom water substantially increased in the cores where ^{13}C-labeled compounds and bicarbonate was added, but not in the background cores (Table 2). In cores from station 2, oxygen penetration depths decreased to 6–9 mm in all incubation experiments except for the core incubated with pyruvate and inhibitor, as well as in cores from station 3, where a decrease in oxygen penetration depth to 8–10 mm was observed (Table 2). In all cores from the three stations, no major differences in IPL-crenarchaeol concentrations upon incubation could be detected (Fig. A1–A3).

The δ^{13}C-values of the biphytanes released from the IPL-GDGTs of the background cores were ranging from −17 to −24 ‰ at all stations (Table 3), which is within the natural variation observed for biphytanes sourced by Thaumarchaeota in marine sediments (Hoefs et al., 1997; Könneke et al., 2012; Schouten et al., 2013). No substantial ^{13}C enrichment of biphytanes was observed in nearly all of the incubation experiments (Fig. 6). The only exception was the tricyclic biphytane in some of the incubations at station 1 (with ^{13}C-labelled substrates bicarbonate, bicarbonate and inhibitor, and pyruvate and inhibitor at nearly all depths) and one incubation (with pyruvate and inhibitor at 6–7 cm depth) at station 2 with enrichments ranging from 2 to 4 ‰. However, due to the large standard deviations in the $\delta^{13}C$ values of the BG cores, only the enrichment of 2–5 ‰ in the 1–2 cm slices of station 1 is statistically significant at a 95 % confidence level ($t(2) = 6.6$–9.0, $P = 0.05$).

To determine whether the added ^{13}C-labelled substrate was incorporated into sedimentary microbial biomass at all, phospholipid-derived fatty acids, stemming from bacteria or eukaryotes, were analyzed from the surface sediments of the bicarbonate, amino acids and glucose incubation experiments. $\delta^{13}C$ values of the most common fatty acids, $C_{16:0}$ and $C_{18:0}$ were enriched in ^{13}C by up to 80 ‰ in the amino acid and glucose incubation experiments compared to the background (Table 4). The cores incubated with ^{13}C-labeled bicarbonate that were analyzed showed no ^{13}C-enriched fatty acids.

4 Discussion

4.1 Biomarkers indicative for live sedimentary Thaumarchaeota

The only biomarker lipids up to now which are probably indicative for live Thaumarchaeota are crenarchaeol IPLs (Pitcher et al., 2011b). Of these, the ones with a glycosidic head group likely contain a substantial fossil component, while the hexose, phosphohexose (HPH)-crenarchaeol is more labile and its concentration corresponds well to DNA-based thaumarchaeal abundance (Pitcher, 2011a, b; Schouten et al., 2012; Lengger et al., 2012a). The presence and dominance of HPH-crenarchaeol in the sediments strongly suggests that live Thaumarchaeota were present in sediments at all the stations (Fig. 5). This was expected for the surface sediment, as oxygen and ammonium were present (Martens-Habbena et al., 2009; Erguder et al., 2009; Hatzenpichler, 2012). The ammonium concentration profiles, with concentrations decreasing upwards, agree with aerobic ammonia oxidation proceeding in the oxygenated parts, using the ammonium diffusing from below. However, HPH-crenarchaeol was present down to 7 cm depth, below the oxygen penetration depth, which could indicate that either live Thaumarchaeota were present at depth, using a metabolic process different from aerobic ammonia oxidation, or that this HPH-crenarchaeol is not stemming from live Thaumarchaeota at depth and was partly preserved. In any case, it is not yet known what exactly the preferred environment for Thaumarchaeota is, as they have been found to be active in low and high ammonia and low and high oxygen environments (Martens-Habbena et al., 2009; Erguder et al., 2009; Hatzenpichler, 2012).

4.2 Activity indicators and label uptake during incubation

Several parameters can be monitored during the incubation experiments to cast a light on sedimentary microbial activities stimulated by the addition of substrates. During incubation, a decrease of oxygen penetration depths would indicate an increased aerobic metabolism at the surface, utilizing oxygen and preventing it from penetrating deeper, which was expected in the slices where organic carbon was added. However, at station 1, this was not observed and oxygen penetration depths only decreased in the background core (Table 2). This could be due to the aeration carried out by bubbling air into the bottom water of the core, and oxygen penetrating deeper into the sandy sediment. However, the cores from station 2 showed a decrease of oxygen penetration depth after incubation, except for the core incubated with pyruvate and inhibitor. Also the cores from station 3 (those where oxygen penetration depth could be measured) showed a decrease, indicating that the oxygen was consumed upon incubation, independent of the type of substrate added to the cores. Ammonia and nitrite concentrations in the bottom water showed no changes during the incubations. No significant differences can be seen if the cores incubated with and without nitrification inhibitor are compared, indicating that either the communities were not affected by it, or potentially that the inhibitor was not as effective in a sedimentary environment as in water incubations (e.g. due to adherence to particles). Nitrate concentrations were, at station 2 and station 3, below detection limit after 6 and 5 days, respectively, suggesting consumption of nitrate (Table 2). This may indicate a strong contribution of denitrification to organic carbon processing in these sediments in the sections below oxygen penetration depth. The increase in $\delta^{13}C$ of the DIC in the bottom water of all cores compared to the background cores (Table 2) clearly showed that the organic substrates added were respired. Previous labeling experiments with similar substrates have been successful in detecting uptake of ^{13}C-label into bacterial PLFA (Guilini et al., 2010), resulting in a highly labeled $C_{16:0}$, among other PLFAs. Indeed, the strong labeling of the bacterial PLFA $C_{16:0}$ at station 3 indicated that the organic substrates were readily taken up by bacteria, and potentially non-photosynthetic eukaryotes (PLFA $C_{16:0}$, $C_{18:0}$), and incorporated into their biomass. Intriguingly, no incorporation was observed upon labeling with bicarbonate, which is in contrast to other studies with oligotrophic sediment cores who used similar concentrations (Guilini et al., 2010). The concentrations of PLFA present might have been too low to allow unambiguous detection of the incorporation in this case.

For archaeal lipids there are no indications of any substantial uptake of ^{13}C-label. IPL-GDGT abundances did not substantially change upon incubation, suggesting that Thaumarchaeota abundances did not change (Fig. 5 showing an average of all incubated cores, with standard deviations). However, this could also indicate that the majority of IPLs in these sediments were fossil (cf. Schouten et al., 2010; Logemann et al., 2011; Lengger et al., 2012a) and not part of living Thaumarchaeota, and thus not affected at all by the incubation. This could include HPH-GDGTs as well, though it is also possible that the bias during analysis, i.e. loss during silica column chromatography (cf. Lengger et al., 2012b), resulted in an underrepresentation of HPH-GDGT-derived biphytanes measured by GC-irMS. Importantly, in nearly all of the incubation experiments, the $\delta^{13}C$ values of all measured biphytanes changed by less than 2‰

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and sometimes even decreased in ^{13}C-content compared to the background values (Fig. 6). In some of the experiments, mainly at station 1, a slight enrichment in the tricyclic biphytane of up to 4 ± 2 ‰ was observed though this difference is not statistically significant for individual experiments. Furthermore, the bicyclic biphytane, also predominantly derived from crenarchaeol, shows in general lower ^{13}C-enrichments than the tricyclic biphytane and also no statistically significant enrichments for individual experiments. Thus, there is no evidence for substantial uptake of ^{13}C-label in archaeal GDGTs, contrary to what is observed for the PLFA.

This apparent lack of uptake could be due to three reasons. Firstly, it is possible that the Thaumarchaeota were active, but the substrates were not taken up. However, in case of the bicarbonate incubations this is unlikely as, in water incubations as well as enrichment cultures – including those of sedimentary Thaumarchaeota – it has been shown to be readily incorporated into biphytanes (Wuchter et al., 2003; Park et al., 2010; Pitcher et al., 2011c). Secondly, it is possible that most of the IPLs measured (i.e. MH- and DH-GDGTs, assuming the chromatographic discrimination against the HPH-GDGTs) are fossil, and thus only a very small amount of these IPLs are part of live and active sedimentary Thaumarchaeota. Finally, it is also possible that a higher proportion of the IPLs are part of sedimentary Thaumarchaeota, but that their metabolism and growth rates are so slow (as opposed to Thaumarchaeota in sea water and enrichment cultures), that no incorporation could be detected over the time scales investigated. These options are discussed in further detail below.

4.3 Estimating growth rates of Thaumarchaeota and turnover rates of IPL-GDGTs

To investigate whether growth rates as well as degradation rates of IPL-GDGTs were responsible for the apparent lack of substantial ^{13}C-labeling, we used data from our experiments to estimate minimal growth rates and the turnover time of IPL-GDGTs. The greatest label incorporation was found at station 1 in the sandy sediment, indicating

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that Thaumarchaeota may have been active there. If a labeling of 2 ‰ of biphytanes is assumed, this can be converted to growth rates by using a formula used for growth of phytoplankton based on the isotope dilution theory of Laws (1984; Eq. 1), with μ representing the growth rate, t_{inc} the incubation time, P^* the atom%-excess of the product, in our case the biphytane, and A^* the atom%-excess of the substrate added (cf. alkenone lipids for eustigmatophyte algae; Popp et al., 2006):

$$\mu = -\frac{1}{t_{inc}} \cdot \ln\left(1 - \frac{P^*}{A^*}\right) \quad (1)$$

Unfortunately, as natural concentrations of the added substrates were not determined, A^* could not be calculated for the incubation experiments with pyruvate, glucose and amino acids. In the bicarbonate experiments, we calculated A^* based on the amount of added 99 %-labeled DIC and natural $\delta^{13}C$ and concentration of DIC (Table 2). For P^* we used the highest enrichment observed at 6–7 cmbsf with no inhibitor, i.e. 4 ‰. This gives an estimate of the maximum μ as $6.3 \times 10^{-4}\ d^{-1}$, corresponding to a generation time of 3 yr. Clearly for the other bicarbonate experiments which resulted in even less label incorporation, generation times will be much higher (e.g. for a 2 ‰ enrichment, 9 yr). Interestingly, growth rates as low as estimated here have been predicted by Valentine (2007), who stated that the archaeal domain, other than bacteria and eukaryotes, has adapted to low energy conditions and is thus characterized by the exceptional ability to live in low energy environments. They are thus not fast in adapting to settings with higher concentrations of available carbon and redox substrates. However, this has not been observed for pelagic Thaumarchaeota as SIP experiments showed a substantial enrichment in ^{13}C in IPL-GDGTs (Wuchter et al., 2003; Pitcher et al., 2011c). Indeed, the generation times calculated from enriched North Sea water labeling experiments conducted by Wuchter et al. (2003; $A^* = 5\%$; $P^* = 0.46\%–400‰$ enrichment) and Pitcher et al. (2011c; $A^* = 9\%$; $P^* = 0.07\%–44‰$ enrichment) were at least an order of magnitude lower than observed here – 50 and 88 d, respectively. Also sedimentary Thaumarchaeota have been shown to incorporate ^{13}C from bicarbonate in

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enrichment cultures, resulting in biphytanes with ^{13}C contents as high as 50 at% (Park et al., 2010).

The calculations above assume exponential growth of sedimentary Thaumarchaeota and an immediate translation of this signal into the lipid pool. If, on the other hand, steady state conditions are assumed, i.e. cell numbers and lipid concentrations are not increasing but remain constant, the turnover time for the sedimentary lipid pool t_{to} can be calculated according to Lin et al. (2013):

$$t_{to} = \frac{A^* \cdot t_{inc}}{P^*} \quad (2)$$

Using Eq. (2), the turnover time of IPL-crenarchaeol in sediments would thus be at least ~ 4 yr based on the 4 ‰ enrichment of the tricyclic biphytane in the bicarbonate incubation experiment. Since labeling was not detected in the other sediments, this turnover time estimate is likely to be a minimal estimate. This large turnover time is not an unusual postulate for thaumarchaeal lipids in sediments, as also Lin et al. (2013) found very low ^{13}C incorporation rates of labeled glucose into sugar moieties of glycosidic GDGTs over > 400 d in slurry incubations of subsurface sediments and used these to estimate turnover times of diglycosyl-lipids of 1700–20 500 yr. Similarly, recently published degradation experiments by Xie et al. (2013) using ^{14}C-labeled IPL-diether lipids found very high turnover times and estimated half-lives of up to 310 kyr under energy-deprived subsurface conditions. This is in strong contrast to GDGTs in the water column: Using the SIP data of Wuchter et al. (2003) and Pitcher et al. (2011c) from incubations of North Sea water, we obtained t_{to} of 76 and 128 d, respectively, for the thaumarchaeal GDGTs.

One possible reason for the slow label incorporation rates is that the incubations were performed shipboard at atmospheric pressure on cores from 200 to 500 m depth and thus the change in the pressure upon recovery of the sediment cores could have caused rupture of the cells. However, if our results are compared to the in situ-incubations carried out by Takano et al. (2010), it is obvious that in situ-incubations did not result in significant incorporation into the biphytanyl chains either. Another possibility, as suggested ibid, is that biphytanyl chains are not or hardly produced by sedimentary Thaumarchaeota, but recycled, and the sugar headgroups and/or the glycerol are only newly generated and ^{13}C-labeled. However, as also the incorporation into the sugar headgroups of GDGTs, as reported by Lin et al. (2013), is similarly low, it suggests that there is indeed a lack of, or only very slow, ^{13}C-uptake into IPL-GDGTs. It seems that stable carbon isotope probing is most likely not the method of choice for thaumarchaeal activity measurements in sediments, where conditions are favorable for preservation of fossil GDGTs, thus creating a large background signal. In the water column, however, where growth of Thaumarchaeota is faster and lipids are likely degraded on shorter time scales, due to the abundant presence of oxygen and lack of mineral matrix protection (Keil et al., 1994; Hedges and Keil, 1995), stable isotope incubations are useful for determining activities of Thaumarchaeota. In sediments that contain a high proportion of fossil IPL-GDGTs which are not part of live Archaea, the incorporation of ^{13}C into the lipids of the few active Thaumarchaeota is probably not enough to change the δ^{13}C of the IPL-GDGTs to a significant degree over the commonly used time scales of SIP experiments.

5 Conclusions

Incubations of sediment cores from the Iceland Shelf were used in order to determine the metabolism of sedimentary Thaumarchaeota. Thaumarchaeal IPLs were present in sediment cores recovered from the Iceland Shelf and the presence of labile IPL-biomarkers for Thaumarchaeota, such as HPH-crenarchaeol, suggested the presence of living Thaumarchaeota. However, incubations with ^{13}C-labeled substrates bicarbonate, pyruvate, glucose and amino acids did not result in any substantial incorporation of the ^{13}C into the biphytanyl chains of these lipids. Turnover times and generation times of thaumarchaeal lipids were estimated to be at least several years suggesting slow growth of sedimentary Thaumarchaeota compared to other sedimentary organ-

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isms, as well as a slow degradation of IPL-GDGTs, in strong contrast to bacterial or eukaryotic PLFAs.

Appendix A

IPL-crenarchaeol profiles show no differences upon incubation at stations 1, 2 and 3 (Figs. A1, A2 and A3, respectively).

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References

Agogué, H., Brink, M., Dinasquet, J., and Herndl, G. J.: Major gradients inputatively nitrifying and non-nitrifying archaea in the deep North Atlantic, Nature, 456, 788–791, 2008.

Alonso-Sáez, L., Waller, A. S., Mende, D. R., Bakker, K., Farnelid, H., Yager, P. L., Lovejoy, C., Tremblay, J.-É., Potvin, M., Heinrich, F., Estrada, M., Riemann, L., Bork, P., Pedrós-Alió, C., and Bertilsson, S.: Role for urea in nitrification by polar marine Archaea, P. Natl. Acd. Sci. USA, 109, 17989–17994, 2012.

Baker, B. J., Lesniewski, R. A., and Dick, G. J.: Genome-enabled transcriptomics reveals archaeal populations that drive nitrification in a deep-sea hydrothermal plume, ISME J., 6, 2269–2279, 2012.

Beman, J. M., Popp, B. N., and Francis, C. A.: Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California, ISME J., 2, 429–441, 2008.

Berg, I. A., Kockelkorn, D., Buckel, W., and Fuchs, G.: A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea, Science, 318, 1782–1786, 2007.

Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sørensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., Sogin, M. L., Brenchley, J. E., Teske, A., House, C. H., and Hinrichs, K.-U.: Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru, P. Natl. Acd. Sci. USA, 103, 3846–3851, 2006.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., and Forterre, P.: Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota, Nat. Rev. Microbiol., 6, 245–252, 2008.

DeLong, E. F.: Archaea in coastal marine environments, P. Natl. Acd. Sci. USA, 89, 5685–5689, 1992.

Erguder, T. H., Boon, N., Wittebolle, L., Marzorati, M., and Verstraete, W.: Environmental factors shaping the ecological niches of ammonia-oxidizing archaea, FEMS Microbiol. Rev., 33, 855–869, 2009.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean, P. Natl. Acd. Sci. USA, 102, 14683–14688, 2005.

Fuhrman, J. A., and Davis, A. A.: Widespread archaea and novel bacteria from the deep sea as shown by 16S rRNA gene sequences, Mar. Ecol.-Prog. Ser., 150, 275–285, 1997.

Grasshoff, K., Kremlin, K., and Ehrhardt, M.: Methods of Seawater Analysis, Verlag Chemie GmbH, Weinheim, 1983.

Guckert, J. B., Antworth, C. P., Nichols, P. D., and White, D. C.: Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments, FEMS Microbiol. Ecol., 31, 147–158, 1985.

Guilini, K., Van Oevelen, D., Soetaert, K., Middelburg, J. J., and Vanreusel, A.: Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: results of an isotope tracer experiment, Limnol. Oceanogr., 55, 1977–1989, 2010.

Hallam, S. J., Konstantinidis, K. T., Putnam, N., Schleper, C., Watanabe, Y., Sugahara, J., Preston, C., de la Torre, J., Richardson, P. M., and DeLong, E. F.: Genomic analysis of the uncultivated marine crenarchaeote *Cenarchaeum symbiosum*, P. Natl. Acd. Sci. USA, 103, 18296–18301, 2006.

Hatzenpichler, R.: Diversity, physiology and niche differentiation of ammonia-oxidizing archaea, Appl. Environ. Microbiol., 78, 7501–7510, 2012.
Hedges, J. I. and Keil, R. G.: Sedimentary organic matter preservation: an assessment and speculative synthesis, Mar. Chem., 49, 81–115, 1995.
Hedges, J. I., Sheng Hu, F., Devol, A. H., Hartnett, H. E., Tsamakis, E., and Keil, R. G.: Sedimentary organic matter preservation: a test for selective degradation under oxic conditions, Am. J. Sci., 299, 529–555, 1999.
Helder, W. and de Vries, R.: An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters, Neth, J. Sea Res., 13, 154–160, 1979.
Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J.: Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean, Appl. Environ. Microbiol., 71, 2303–2309, 2005.
Hershberger, K. L., Barns, S. M., Reysenbach, A.-L., Dawson, S. C., and Pace, N. R.: Wide diversity of Crenarchaeota, Nature, 384, p. 420, 1996.
Hoefs, M. J. L., Schouten, S., de Leeuw, J. W., King, L. L., Wakeham, S. G., and Sinninghe Damsté, J. S.: Ether Lipids of Planktonic Archaea in the Marine Water Column, Appl. Environ. Microbiol., 63, 3090–3095, 1997.
Huguet, C., Hopmans, E. C., Febo-Ayala, W., Thompson, D. H., Sinninghe Damsté, J. S., and Schouten, S.: An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids, Org. Geochem., 37, 1036–1041, 2006.
Ingalls, A. E., Shah, S. R., Hansman, R. L., Aluwihare, L. I., Santos, G. M., Druffel, E. R. M., and Pearson, A.: Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon, P. Natl. Acd. Sci. USA, 103, 6442–6447, 2006.
Jung, M. Y., Park, S.-J., Min, D., Kim, J.-S., Rijpstra, W. I. C., Sinninghe Damsté, J. S., Kim, G.-J., Madsen, E. L., and Rhee, S.-K.: Enrichment and Characterization of an autotrophic ammonia-oxidizing archaeon of mesophilic crenarchaeal group I.1a from an agricultural soil, Appl. Environ. Microbiol., 77, 8635–8647, 2011.
Kaneko, M., Kitajima, F., and Naraoka, H.: Stable hydrogen isotope measurement of archaeal ether-bound hydrocarbons, Org. Geochem., 42, 166–172, 2011.
Karner, M. B., DeLong, E. F., and Karl, D. M.: Archaeal dominance in the mesopelagic zone of the Pacific Ocean, Nature, 409, 507–510, 2001.
Keil, R. G., Montluçon, D. B., Prahl, F. G., and Hedges, J. I.: Sorptive preservation of labile organic matter in marine-sediments, Nature, 370, 549–552, 1994.

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Lack of ^{13}C-label incorporation
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Kim, J.-G., Jung, M.-Y., Park, S.-J., Rijpstra, W. I. C., Sinninghe Damsté, J. S., Madsen, E. L., Min, D., Kim, J.-S., Kim, G.-J., and Rhee, S.-K.: Cultivation of a highly enriched ammonia-oxidizing archaeon of thaumarchaeotal group I.1b from an agricultural soil, Environ. Microbiol., 14, 1528–1543, 2012.
Koga, Y. and Nakano, M.: A dendrogram of archaea based on lipid component parts composition and its relationship to rRNA phylogeny, Syst. Appl. Microbiol., 31, 169–182, 2008.
Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A.: Isolation of an autotrophic ammonia-oxidizing marine archaeon, Nature, 437, 543–546, 2005.
Könneke, M., Lipp, J. S., and Hinrichs, K.-U.: Carbon isotope fractionation by the marine ammonia-oxidizing archaeon Nitrosopumilus maritimus, Org. Geochem., 48, 21–24, 2012.
Laws, E. A.: Improved estimates of Phytoplankton Carbon Based on ^{14}C Incorporation into Chlorophyll *a*, J. Theor. Biol., 110, 425–434, 1984.
Lengger, S. K., Hopmans, E. C., Reichart, G.-J., Nierop, K. G. J., Sinninghe Damsté, J. S., and Schouten, S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone: II. Selective preservation and degradation in sediments and consequences for the TEX_{86}, Geochim. Cosmochim. Ac., 98, 244–258, 2012a.
Lengger, S. K., Hopmans, E. C., Sinninghe Damsté, J. S., and Schouten, S.: Comparison of extraction and work up techniques for analysis of core and intact polar tetraether lipids from sedimentary environments, Org. Geochem., 47, 34–40, 2012b.
Lin, Y. S., Lipp, J. S., Elvert, M., Holler, T., and Hinrichs, K.-U.: Assessing production of the ubiquitous archaeal diglycosyl tetraether lipids in marine subsurface sediment using intramolecular stable isotope probing, Environ. Microbiol., 15, 1634–1646, 2013.
Lipp, J. S. and Hinrichs, K.-U.: Structural diversity and fate of intact polar lipids in marine sediments, Geochim. Cosmochim. Ac., 73, 6816–6833, 2009.
Lipp, J. S., Morono, Y., Inagaki, F., and Hinrichs, K.-U.: Significant contribution of Archaea to extant biomass in marine subsurface sediments, Nature, 454, 991–994, 2008.
Logemann, J., Graue, J., Köster, J., Engelen, B., Rullkötter, J., and Cypionka, H.: A laboratory experiment of intact polar lipid degradation in sandy sediments, Biogeosciences, 8, 2547–2560, doi:10.5194/bg-8-2547-2011, 2011.
Martens-Habbena, W., Berube, P. M., Urakawa, H., de la Torre, J. R., and Stahl, D. A.: Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria, Nature, 461, 976–979, 2009.

BGD
10, 12807–12847, 2013
Lack of ^{13}C-label incorporation
S. K. Lengger et al.

Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate, Anal. Chim. Acta, 27, 31–36, 1962.
Mußmann, M., Brito, I., Pitcher, A., Sinninghe Damsté, J. S., Hatzenpichler, R., Richter, A., Nielsen, J. L., Nielsen, P. H., Müller, A., Daims, H., Wagner, M., and Head, I. M.: Thaumarchaeotes abundant in refinery nitrifying sludges express amoA but are not obligate autotrophic ammonia oxidizers, P. Natl. Acd. Sci. USA, 108, 16771–16776, 2011.
Ouverney, C. C. and Fuhrman, J. A.: Marine planktonic archaea take up amino acids, Appl. Environ. Microbiol., 66, 4829–4833, 2000.
Park, B.-J., Park, S.-J., Yoon, D.-N., Schouten, S., Sinninghe Damsté, J. S., and Rhee, S.-K.: Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria, Appl. Environ. Microbiol., 76, 7575–7587, 2010.
Pitcher, A., Hopmans, E. C., Schouten, S., and Sinninghe Damsté, J. S.: Separation of core and intact polar archaeal tetraether lipids using silica columns: insights into living and fossil biomass contributions, Org. Geochem., 40, 12–19, 2009.
Pitcher, A., Hopmans, E. C., Mosier, A. C., Park, S.-J., Rhee, S.-K., Francis, C. A., Schouten, S., and Sinninghe Damsté, J. S.: Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing archaea enriched from marine and estuarine sediments, Appl. Environ. Microbiol., 77, 3468–3477, 2011a.
Pitcher, A., Hopmans, E. C., Villanueva, L., Reichart, G.-J., Schouten, S., and Sinninghe Damsté, J. S.: Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the arabian sea oxygen minimum zone, ISME J., 5, 1896–1904, 2011b.
Pitcher, A., Wuchter, C., Siedenberg, K., Schouten, S., and Sinninghe Damsté, J. S.: Crenarchaeol tracks winter blooms of ammonia-oxidizing Thaumarchaeota in the coastal North Sea, Limnol. Oceanogr., 56, 2308–2318, 2011c.
Popp, B. N., Bidigare, R. R., Deschenes, B., Laws, E. A., Prahl, F. G., Tanimoto, J. K., and Wallsgrove, R. J.: A new method for estimating growth rates of alkenone-producing haptophytes, Limnol. Oceanogr., 4, 114–129, 2006.
Revsbech, N. P.: An oxygen microsensor with a guard cathode, Limnol. Oceanogr., 34, 474–478, 1989.
Schouten, S., Hoefs, M. J. L., Koopmans, M. P., Bosch, H.-J., and Sinninghe Damsté, 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.

Schouten, S., Hopmans, E. C., Baas, M., Boumann, H., Standfest, S., Könneke, M., Stahl, D. A., and Sinninghe Damsté, J. S.: Intact membrane lipids of "*Candidatus* Nitrosopumilus maritimus", a cultivated representative of the cosmopolitan mesophilic group I crenarchaeota, Appl. Environ. Microbiol., 74, 2433–2440, 2008.

Schouten, S., Middelburg, J. J., Hopmans, E. C., and Sinninghe Damsté, J. S.: Fossilization and degradation of intact polar lipids in deep subsurface sediments: a theoretical approach, Geochim. Cosmochim. Acta, 74, 3806–3814, 2010.

Schouten, S., Pitcher, A., Hopmans, E. C., Villanueva, L., van Bleijswijk, J., and Sinninghe Damsté, J. S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone: I. Selective preservation and degradation in the water column and consequences for the TEX_{86}, Geochim. Cosmochim. Ac., 98, 228–243, 2012.

Schouten, S., Hopmans, E. C., and Sinninghe Damsté, J. S.: The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review, Org. Geochem., 54, 19–61, 2013.

Sinninghe Damsté, J. S., Schouten, S., Hopmans, E. C., van Duin, A. C. T., and Geenevasen, J. A. J.: Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota, J. Lipid Res., 43, 1641–1651, 2002.

Sinninghe Damsté, J. S., Rijpstra, W. I. C., Hopmans, E. C., Jung, M. Y., Kim, J. G., Rhee, S. K., Stieglmeier, M., and Schleper, C.: Intact polar and core dibiphytanyl glycerol tetraether lipids of group I.1a and I.1b thaumarchaeota in soil, Appl. Environ. Microbiol., 78, 6866–6874, 2012.

Spang, A., Hatzenpichler, R., Brochier-Armanet, C., Rattei, T., Tischler, P., Spieck, E., Streit, W., Stahl, D. A., Wagner, M., and Schleper, C.: Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum Thaumarchaeota, Trends Microbiol., 18, 331–340, 2010.

Takano, Y., Chikaraishi, Y., Ogawa, N. O., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs, K.-U., and Ohkouchi, N.: Sedimentary membrane lipids recycled by deep-sea benthic archaea, Nat. Geosci., 3, 858–861, 2010.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A., and Schleper, C.: Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil, P. Natl. Acd. Sci. USA, 108, 8420–8425, 2011.

Valentine, D. L.: Adaptations to energy stress dictate the ecology and evolution of the Archaea, Nat. Rev. Microbiol., 5, 316–323, 2007.

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Walker, C. B., de la Torre, J. R., Klotz, M. G., Urakawa, H., Pinel, N., Arp, D. J., Brochier-Armanet, C., Chain, P. S. G., Chan, P. P., Gollabgir, A., Hemp, J., Hügler, M., Könneke, M., Shin, M., Lawton, T. J., Lowe, T., Martens-Habbena, W., Sayavedra-Soto, L. A., Lang, D., Sievert, S. M., Rosenzweig, A. C., Manning, G., and Stahl, D. A.: Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea, P. Natl. Acd. Sci. USA, 107, 8818–8823, 2010.
Wuchter, C., Schouten, S., Boschker, H. T. S., and Sinninghe Damsté, J. S.: Bicarbonate uptake by marine Crenarchaeota, FEMS Microbiol. Lett., 219, 203–207, 2003.
Xie, S., Lipp, J. S., Wegener, G., Ferdelman, T. G., and Hinrichs, K.-U.: Turnover of microbial lipids in the deep biosphere and growth of benthic archaeal populations, P. Natl. Acd. Sci. USA, in press, 2013.

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Table 1. $\delta^{13}C$ values of the acyclic, bicyclic, and tricyclic biphytanes obtained via H_2/PtO_2 and $LiAlH_4$ reduction of alkyliodides formed via HI ether cleavage.

	$\delta^{13}C$ (‰)					
	acyclic		bicyclic		tricyclic	
Sample	H_2/PtO_2	$LiAlH_4$	H_2/PtO_2	$LiAlH_4$	H_2/PtO_2	$LiAlH_4$
Station 1 0–1 cm	−20.3 ± 0.4	−20.2 ± 0.0	−19.7 ± 0.7	−20.0 ± 0.6	−19.5 ± 1.1	−21.2 ± 0.8
Station 1 1–2 cm	−19.9 ± 0.6	−22.0 ± 0.2	−18.4 ± 0.2	−20.2 ± 1.6	−19.0 ± 0.2	−19.8 ± 0.6
Station 2 0–1 cm	−22.7 ± 0.7	−22.0 ± 0.4	−24.2 ± 1.0	−22.4 ± 0.3	−22.6 ± 1.6	−22.0 ± 0.4
Station 2 1–2 cm	−24.0 ± 1.0	−25.6 ± 0.2	−22.3 ± 1.4	−25.6 ± 0.8	−22.6 ± 0.2	−25.0 ± 0.6

	Total amount µg					
	acyclic		bicyclic		tricyclic	
Sample	H_2/PtO_2	$LiAlH_4$	H_2/PtO_2	$LiAlH_4$	H_2/PtO_2	$LiAlH_4$
Station 1 0–1 cm	0.79	0.28	0.27	0.16	0.31	0.18
Station 1 1–2 cm	0.42	0.16	0.14	0.07	0.15	0.08
Station 2 0–1 cm	0.45	0.34	0.17	0.16	0.23	0.23
Station 2 1–2 cm	0.13	0.05	0.04	0.0	0.06	0.0

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Table 2. The bottom water NO_3^- concentrations and δ^{13}DIC changes over the incubation time (Δ_{t-t_0}), as well as oxygen penetration depths measured after the incubations. In brackets, the average values of oxygen penetration depth for the cores before incubation. BG ... background, Bic ... bicarbonate, Pyr ... pyruvate, +Inh ... nitrification inhibitor nitrapyrine added.

Station	Incubation	NO_3^- [µmol L^{-1}] $t = 0$ h	$t = 62$ h	$t = 144$ h	$\Delta\delta^{13}DIC_{t-t_0}$ [‰] $t = 62$ h	$t = 144$ h	O_2 Pen depth [mm] $t = 144$ h
							(before: 17)
1	BG	17.9	19.1	4.1	0.6	2.8	11
	Bic	15.9	16.2	8.2	66.5	156.2	16
	Pyr	18.1	19.2	5.9	109.1	457.0	16
	BG+Inh	15.5	16.3	13.9	−0.5	2.6	20
	Bic+Inh	11.5	12.0	3.8	135.3	275.5	20
	Pyr+Inh	25.4	25.6	14.3	72.7	671.1	16
							(before: 13)
2	BG	12.14	11.37	0.09	−0.5	2.9	9
	Bic	5.55	4.05	0.07	336.5	606.0	6
	Pyr	13.77	12.48	0.12	366.6	1570.7	6
	BG+Inh	10.72	10.45	0.19	0.1	3.5	5
	Bic+Inh	7.22	6.79	0.09	255.4	570.1	6
	Pyr+Inh	5.01	3.17	0.06	511.3	1136.4	16
							(before: 16)
3	BG	13.84	0.00	0.13	1.5	−2.3	n.d.*
	Bic	13.83	12.60	0.08	558.2	814.3	n.d.*
	Pyr	6.45	4.23	0.12	1108.9	1496.3	n.d.*
	Glu	14.14	12.03	0.11	90.4	194.2	n.d.*
	AA	15.34	13.55	0.15	82.7	201.9	n.d.*
	BG+Inh	16.06	13.39	0.11	0.3	4.9	8
	Bic+Inh	15.23	14.05	0.18	244.8	520.3	n.d.*
	Pyr+Inh	8.92	6.68	0.10	682.7	1644.5	8
	Glu+Inh	5.86	4.77	0.03	110.8	301.2	10
	AA+Inh	14.36	13.41	0.09	62.2	368.2	n.d.*

* n.d. – could not be determined.

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Table 3. Background (BG) values of δ^{13}C of the biphytanes measured in the sediment, i.e. δ^{13}C of the biphytanes that were incubated just with water, but under the same conditions. These values were used for determination of the $\Delta\delta^{13}$C: BG values were subtracted from the δ^{13}C of the corresponding value measured from the cores incubated with label (Fig. 6). Numbers indicate the depth interval the sample was coming from, +: nitrification inhibitor nitrapyrine added for incubation, −: no inhibitor added. Values at station 2 could not be determined due to low concentrations.

		Station 1 δ^{13}C [‰]			Station 2 δ^{13}C [‰]			Station 3 δ^{13}C [‰]		
	Sediment (cmbsf)	acyclic BP	bicyclic BP	tricyclic BP	acyclic BP	bicyclic BP	tricyclic BP	acyclic BP	bicyclic BP	tricyclic BP
−	0–1	−22.0 ± 0.1	−20.5 ± 1.1	−19.9 ± 0.3	n.d.*	n.d.*	n.d.*	−20.4 ± 0.2	−20.2 ± 1.2	−19.8 ± 0.8
	1–2	−21.4 ± 0.5	−20.7 ± 0.1	−20.2 ± 0.0	n.d.*	n.d.*	n.d.*	−19.6 ± 1.2	−18.3 ± 1.4	−17.7 ± 1.9
	6–7	−21.4 ± 0.2	−22.4 ± 1.9	−21.5 ± 2.3	n.d.*	n.d.*	n.d.*	−20.6 ± 0.4	−20.1 ± 0.4	−19.2 ± 0.6
+	0–1	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	−19.3 ± 0.6	−19.7 ± 0.6	−19.4 ± 0.3
	1–2	−22.0 ± 0	−21.2 ± 0.4	−21.3 ± 0	−22.9 ± 0.9	−21.4 ± 1.4	−20.3 ± 0.9	−19.4 ± 0.1	−19.9 ± 0.4	−19.2 ± 0.2
	6–7	−22.4 ± 0	−20.0 ± 0.6	−22.2 ± 0	−22.0 ± 0.0	−21.0 ± 0.4	−20.0 ± 0.4	−22.4 ± 0.0	−21.4 ± 0.4	−20.7 ± 0.1

* n.d. – could not be determined.

Table 4. $\delta^{13}C$ values of fatty acids measured in the 0–1 cm slice in cores from station 3, incubated without inhibitor, for 5 days.

Fatty acid	$\delta^{13}C$ [‰] BG	Bic	Glu	AA
$C_{16:0}$	−25.5 ± 0.2	−25.3 ± 0.6	20.6 ± 0.5	30.0 ± 3.9
$C_{18:0}$	−24.6*	−24.5 ± 0.2	66.2 ± 0.4	63.1 ± 2.1

* Single measurement.

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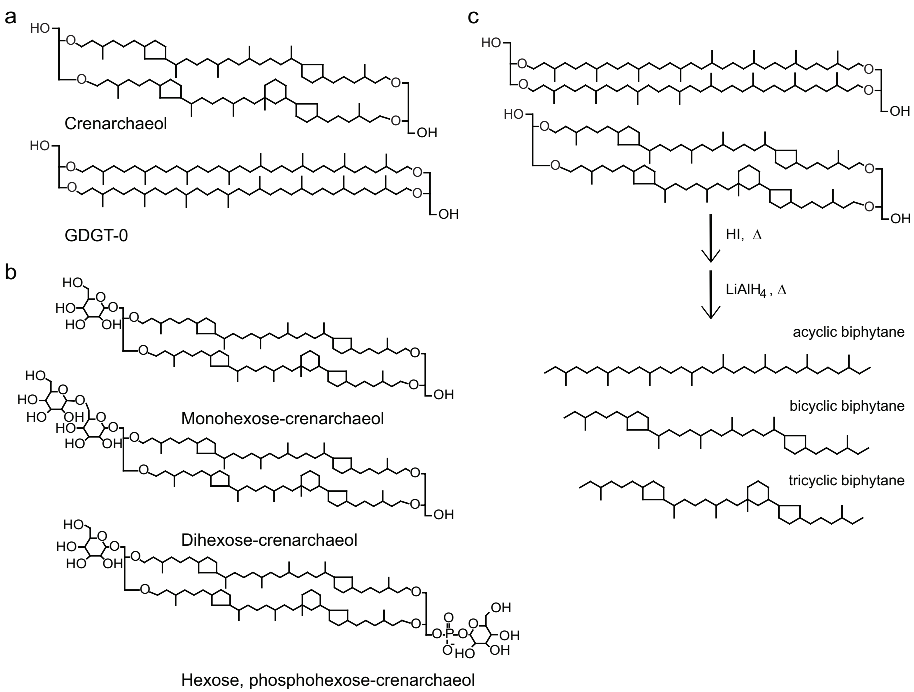

Fig. 1. Structures of crenarchaeol and GDGT-0 **(a)**, IPL-crenarchaeol species **(b)** and biphytanyl chains released from crenarchaeol and GDGT-0 by chemical degradation **(c)**.

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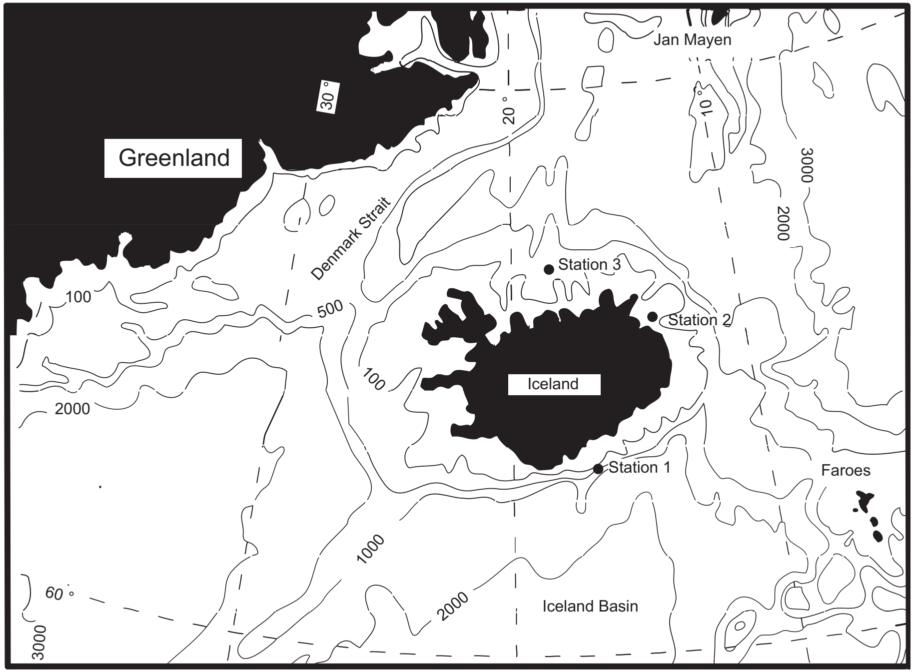

Fig. 2. Map of the sampling stations (1, 2 and 3) on the Iceland shelf.

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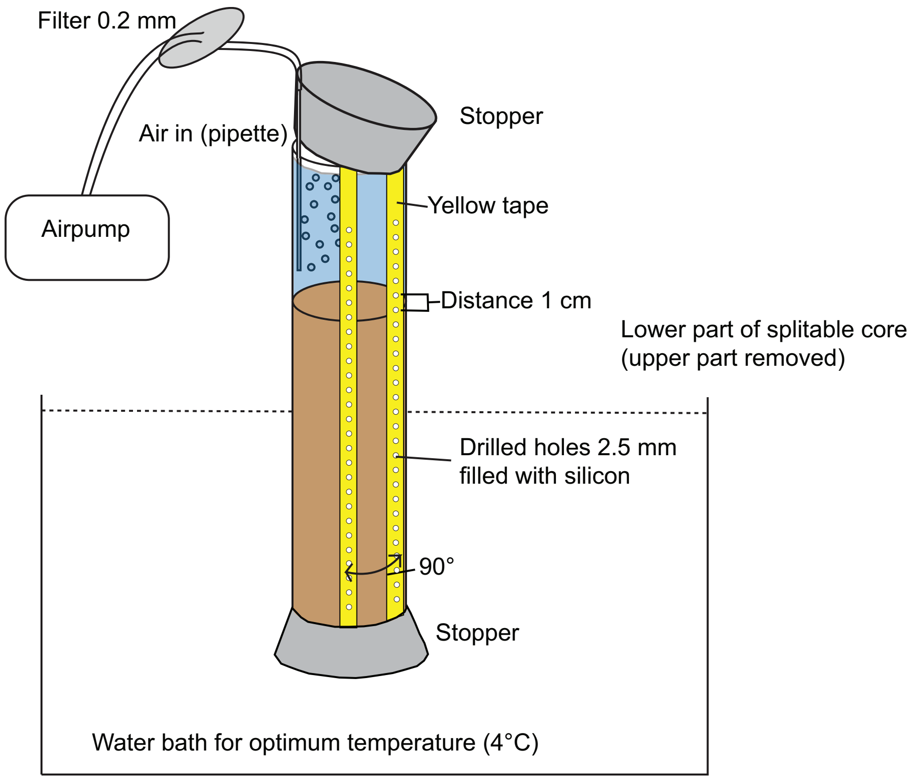

Fig. 3. Scheme of the incubation set-up of the cores.

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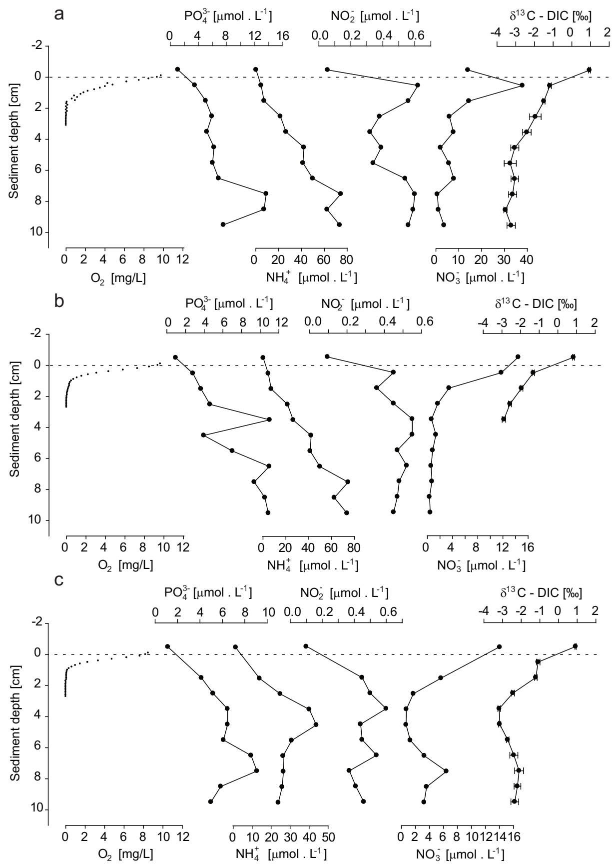

Fig. 4. Oxygen microprofiles and pore water results vs. depth at station 1 **(a)**, 2 **(b)** and 3 **(c)**.

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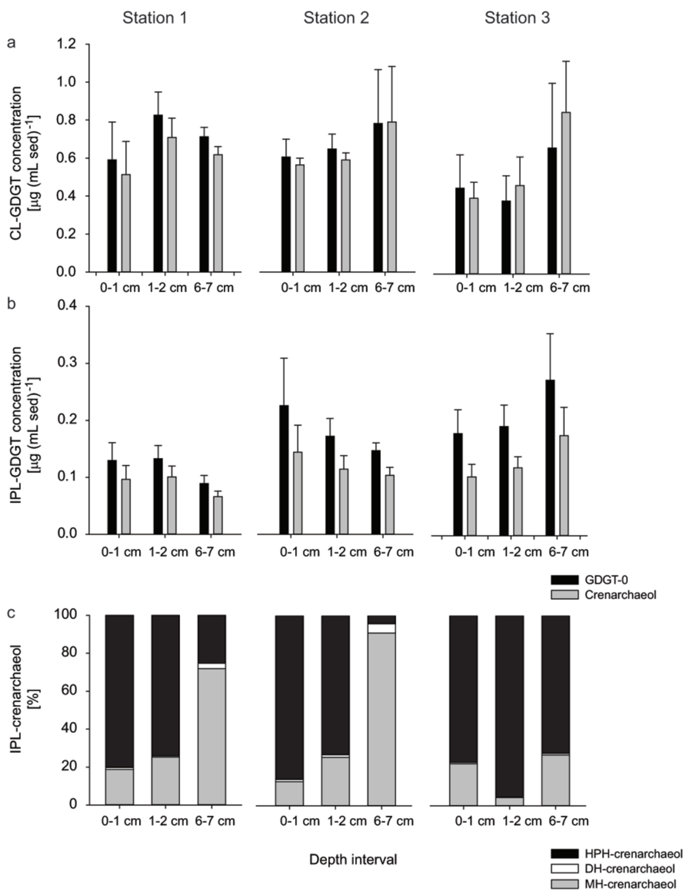

Fig. 5. GDGT-0 and crenarchaeol concentrations (averages), for CL-GDGTs **(a)** and IPL-derived GDGTs **(b)** at all three stations and core depths. Proportions of head groups of IPL-crenarchaeol at all three stations **(c)**.

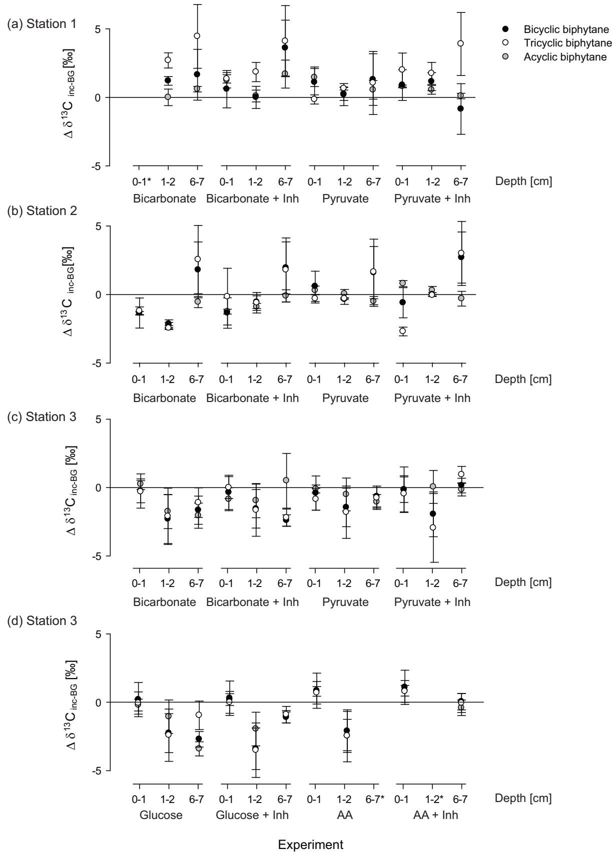

Fig. 6. Difference in δ^{13}C-values ($\Delta\delta^{13}C_{inc\text{-}bg}$) of the acyclic, bicyclic and tricyclic biphytanes in ‰ in the cores incubated with labeled substrate vs. the δ^{13}C-values in the background cores (incubated with and without inhibitor) at station 1 **(a)**, station 2 **(b)** and station 3 **(c)** from the cores incubated with bicarbonate and pyruvate, with and without inhibitor, and the cores incubated with glucose and amino acids with and without inhibitor, at station 3 **(d)**.

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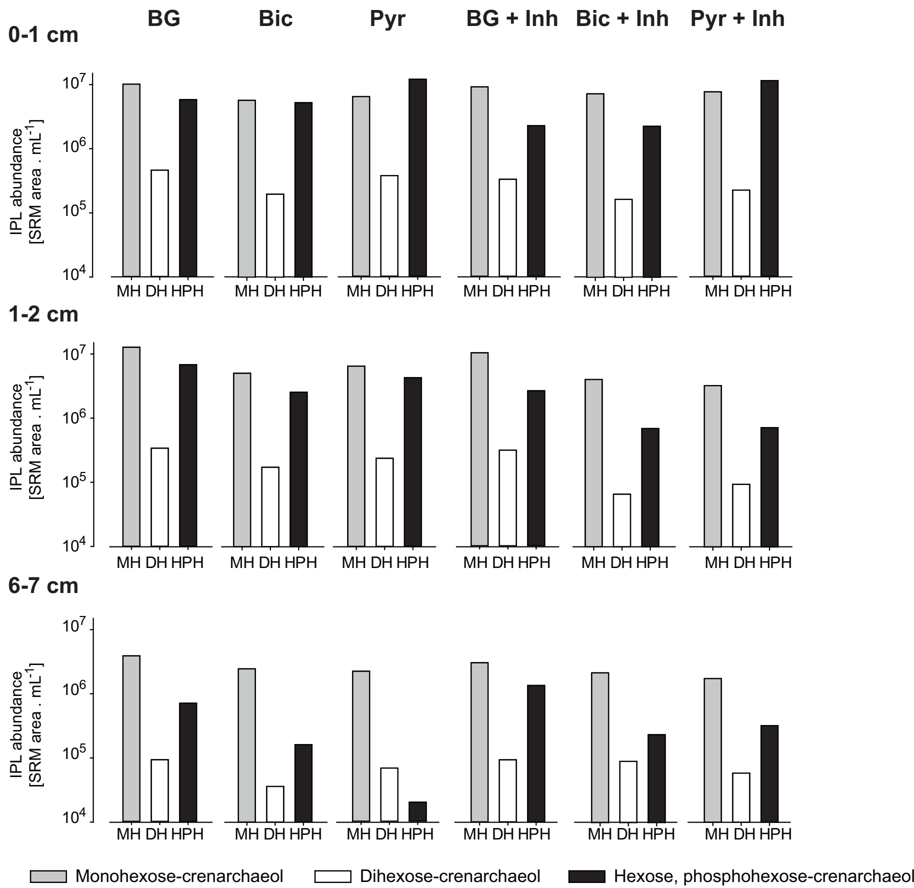

Fig. A1. IPL-crenarchaeol profiles of the incubated cores – background (BG), bicarbonate (Bic) and pyruvate (Pyr) without (left plot) and with nitrification inhibitor (right plot). Top graphs shows the values in SRM area mL^{-1} from 0–1 cm depth, graphs in the middle the profiles from 1–2 cm depth and at the bottom the profiles from 6–7 cm depth.

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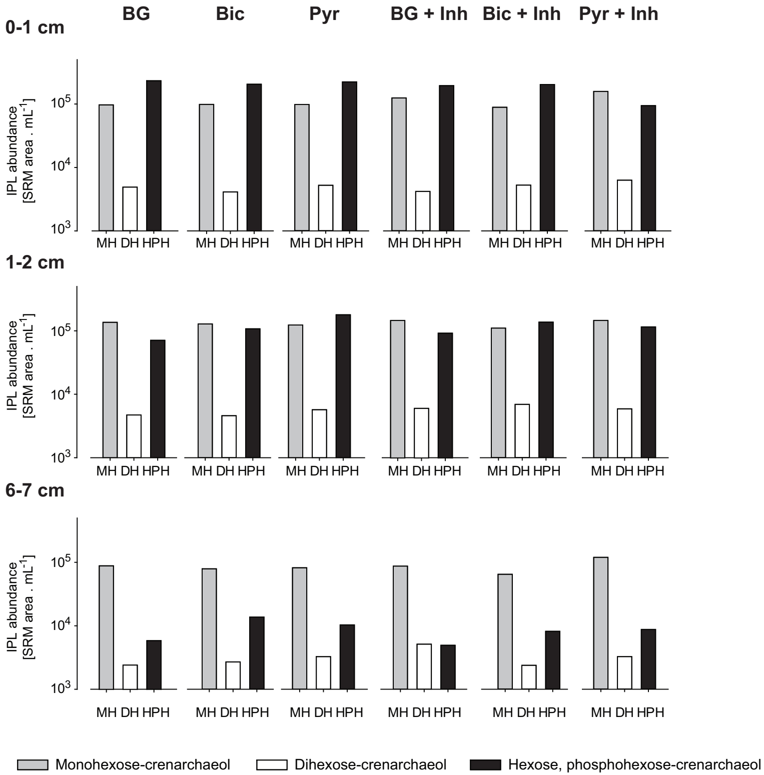

Fig. A2. IPL-crenarchaeol profiles of the incubated cores – background (BG), bicarbonate (Bic) and pyruvate (Pyr) without (left plot) and with nitrification inhibitor (right plot). Top graphs shows the values in SRM area mL^{-1} from 0–1 cm depth, graphs in the middle the profiles from 1–2 cm depth and at the bottom the profiles from 6–7 cm depth at station 2.

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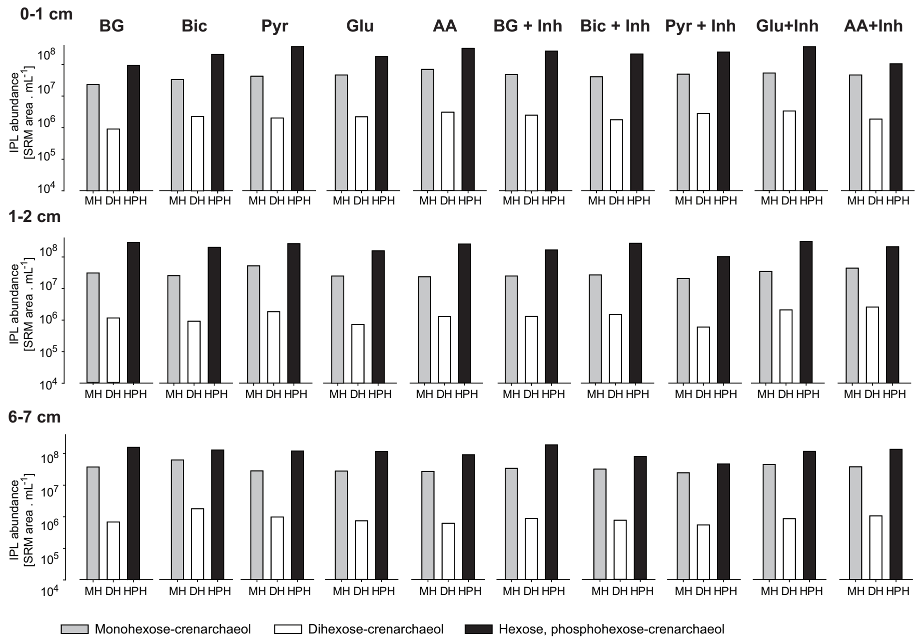

Fig. A3. IPL-crenarchaeol profiles of the incubated cores – background (BG), bicarbonate (Bic), pyruvate (Pyr), glucose (Glu) and amino acids (AA) without (left plot) and with nitrification inhibitor (right plot). Top graphs shows the values in SRM area mL^{-1} from 0–1 cm depth, graphs in the middle the profiles from 1–2 cm depth and at the bottom the profiles from 6–7 cm depth at station 3.

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