Himmler, T., et al., 2021, Putative fossils of chemotrophic microbes preserved in seep carbonates from Vestnesa Ridge, off northwest Svalbard, Norway: Geology, https://doi.org/10.1130/G49620.1.

SUPPLEMENTAL MATERIAL

METHODS

Thin section microscopy

Polished thin sections (~30 µm thickness, 6.5 x 5 cm) were prepared from carbonate slabs embedded in epoxy resin and were examined using scanning electron microscopy (Zeiss Leo 1450 VP; 15 kV; ~11 and ~15 mm working distance; variable pressure) and transmitted light microscopy (Zeiss Axioplan2 equipped with an AxioCam ERc 5s digital camera; Figs. S1 and S4).

Thin section surface polishing

Thin section surfaces with abundant clustered microstructures were polished using a Leica EM RES102 argon (Ar) ion milling system, applying an Ar plasma at 8 kV for approximately 1 hour (3 degrees incidence angle) for surface leveling, followed by milling at 4 kV for approximately 1 hour (15 degrees incidence angle) for cleaning (Fig. S4).

Mineralogy

Quantitative mineralogical composition was determined by X-ray diffraction on powders obtained with a hand-held microdrill from cut slab surfaces (Data file S1). Powders were analyzed using a Bruker D8 Advance diffractometer using copper K-α radiation at a 2Θ scanning angle of 3° to 75° (step size of 0.02°, 1 s per step). Minerals were identified by automatic and manual peak search using the Bruker Diffrac EVA 5.2 software; quantification was performed applying Rietveld refinement using TOPAS 5.0 software (2 to 3 weight-% uncertainty).

Lipid biomarker and compound-specific carbon isotope analyses

Two cut slabs of carbonate samples P1606001 and P1606002 were prepared for lipid biomarker analysis. The slabs were cleaned with deionised water and the exterior surfaces were removed with hammer and chisel. The remaining sample material was cleaned with acetone and crushed to small nugget-sized pieces. Dissolution of the carbonate matrix of the nugget-sized pieces was done by slowly pouring 10% hydrochloric acid onto them. After ca. 75% of the carbonate was dissolved, the hydrochloric acid solution was discarded. The remaining sediment was collected, saponified and extracted. To release ester-bond carboxylic acids, the sample was

treated with 6% KOH in methanol (base hydrolysis). The reaction was done in a 100 ml screw-cap vial in an ultrasonic bath at 80°C for 2 hours. The saponification extract was collected in a separatory funnel. In the following, the samples were extracted three times with a mixture of dichloromethane and methanol (3:1). The samples were ultrasonicated for 15 minutes at ambient temperature. After each extraction, the dichloromethane and methanol mixture was combined with the saponification extract in the separatory funnel. Then, demineralized water was added, the aqueous phase was acidified to release the fatty acid salts and transfer them as free fatty acids to the organic phase. The combined total lipid extract (TLE) was collected in a round flask and dried to near dryness with a rotation evaporator. An aliquot of the TLE was further separated in *n*-hexane soluble maltenes and dichloromethane-soluble asphaltenes. The maltenes were separated with an aminopropyl-modified silica gel column into four fractions with increasing polarity (Birgel et al., 2008). Only hydrocarbon, alcohol, and carboxylic acid fractions contained indigenous compounds, the ketone fraction is not further discussed. Alcohols and carboxylic acids were derivatized with N,O-bis(trimethylsilyl)fluoracetamide (BSTFA) and pyridine (1:1) and 10% BF_3 in methanol for 1 hour at 80°C, respectively. All samples were analyzed with a GC-MS (Thermo Electron Trace GC Ultra) coupled to a Thermo Electron DSQ II mass spectrometer with a DB-5 MS ultra inert column (30 m; 0.25 mm inner diameter, 0.25 mm film thickness). The temperature program was 50°C (3 min), then with 8°C/min to 325°C. The final temperature was held for 20 minutes. Carrier gas on the GC-MS was helium. Compound-specific carbon isotopes were measured on a gas chromatograph (Agilent 6890) coupled with a Thermo Finnigan Combustion III interface to a Finnigan Delta Plus XL isotope ratio mass spectrometer (GC-IRMS). The GC conditions were identical to those of the GC-MS. The alcohols and carboxylic acids were corrected for the addition of carbon atoms during derivatization. The samples were measured in duplicate and the standard deviation of the isotope measurements was below 0.8‰ and was determined with a Schimmelmann reference Standard Mixture B. Quantification of compounds was done via internal standards added prior saponification.

Focused ion beam–transmission electron microscopy (FIB–TEM)

FIB-foils for TEM analyses were prepared under ultrahigh vacuum oil-free conditions using the FEI FIB 200TEM instrument at the GeoForschungsZentrum Potsdam, Germany. The foils are approximately 15×10×0.150 µm (length, height, thickness) in dimension. Foils were milled from a thin section and a polished rock sample with a gallium (Ga^{2+}) ion beam at 30 kV acceleration voltage. Subsequently, the foils were placed on a holey carbon film which was

mounted on a copper grid sample holder. Carbon coating was omitted. Electron conductivity was induced by implantation of a thin layer of Ga^{2+}–ions into the sample surface (Wirth, 2009). Prior to TEM analyses potential surface contaminations were removed using a plasma (25 vol.% oxygen, 75 vol.% argon) cleaner for about 10 seconds. TEM was carried out on a FEI Tecnai G2 F20 X-Twin transmission electron microscope. Elemental compositions were measured with an EDX energy dispersive X-ray spectroscopy system.

Nanoscale secondary ion mass spectrometry (nanoSIMS) $\delta^{13}C$ analyses

The nanoSIMS $\delta^{13}C$ analyses were done on a gold-coated (~30 nm) TEM-foil milled from a thin section, using the CAMECA NanoSIMS 50L instrument at the Division of Geological and Planetary Sciences, California Institute of Technology, U.S.A. Prior to measurements, areas of interest were sputtered using a primary cesium (Cs^+) beam for approximately five minutes to locally remove the coating. Signals of $^{12}C^-$, $^{13}C^-$, and $^{40}Ca^{2+}$ ions were collected on electron multipliers with the electron gun for charge compensation, at a stable primary column current (current not recorded) over 1x1 and 2x2 µm square regions on the sample and the standard material (Data file S1). Ceramacast 905 (C-905), a synthetic standard containing organic carbon in a silicate matrix (House, 2015), was repeatedly measured before and after sample analyses. Average uncertainty for individual $\delta^{13}C$ analyses (1σ) was calculated at 5.3‰ (n = 6; Data file S1).

During standard measurements monitoring $^{13}C/^{12}C$ and ^{12}C counts, a positive linear correlation was observed (Fig. S2A). The correlation suggests a quasi-simultaneous arrival (QSA) effect. The QSA effect occurs when a single incoming primary ion produces multiple secondary ions that are recorded by the electron multipliers as a single event (Slodzian et al., 2004). As a result, the recorded counts for the major isotope (i.e. ^{12}C) are lower than the actual emitted secondary $^{12}C^-$ ions and thus the calculated $^{13}C/^{12}C$ ratio becomes artificially higher with increasing counts. In order to rule out other potential analytical artifacts influencing $^{13}C/^{12}C$ and ^{12}C signal relationships, such as heterogeneity of C-905, a homogeneous graphite standard (unknown $\delta^{13}C$) was studied in a follow-up session. By changing the slit aperture at a constant primary column current (25 nA), a similar positive linear correlation was observed (Fig. S2B).

Correction for QSA was done following Slodzian et al. (2004) (excluding 2σ outliers) and instrumental mass fractionation (IMF) was determined on the working standard (N = 22) using the following equation:

$$\alpha_{IMF} = \frac{(1000 + \delta^{13}C_{QSA})}{(1000 + \delta^{13}C_{true})}$$

where $\delta^{13}C_{QSA}$ is the QSA-corrected value and $\delta^{13}C_{true}$ was assumed −27.9‰ VPDB for the standard material (House, 2015). The calculated α_{IMF} is 0.963 (σ_{IMF} = 3.4‰, n=8). This IMF was then applied to correct each individual sample analysis using the same equation. The total uncertainty for each measurement is calculated as following (House et al., 2013)

$$\sigma_{tot.} = \sqrt{\sigma_{\int.}^2 + \sigma_{IMF}^2}$$

where $\sigma_{int.}$ is the internal error of each analysis spot and σ_{IMF} is the mean IMF standard error calculated after QSA correction. Uncertainties for sample $\delta^{13}C$ analyses (1σ) ranged from 3.9 to 8.1 ‰, averaging at 5.1‰ (n = 6; Data file S1). Figure S3 compares $\delta^{13}C$ values corrected and non-corrected for QSA effect.

SUPPLEMENTAL FIGURES

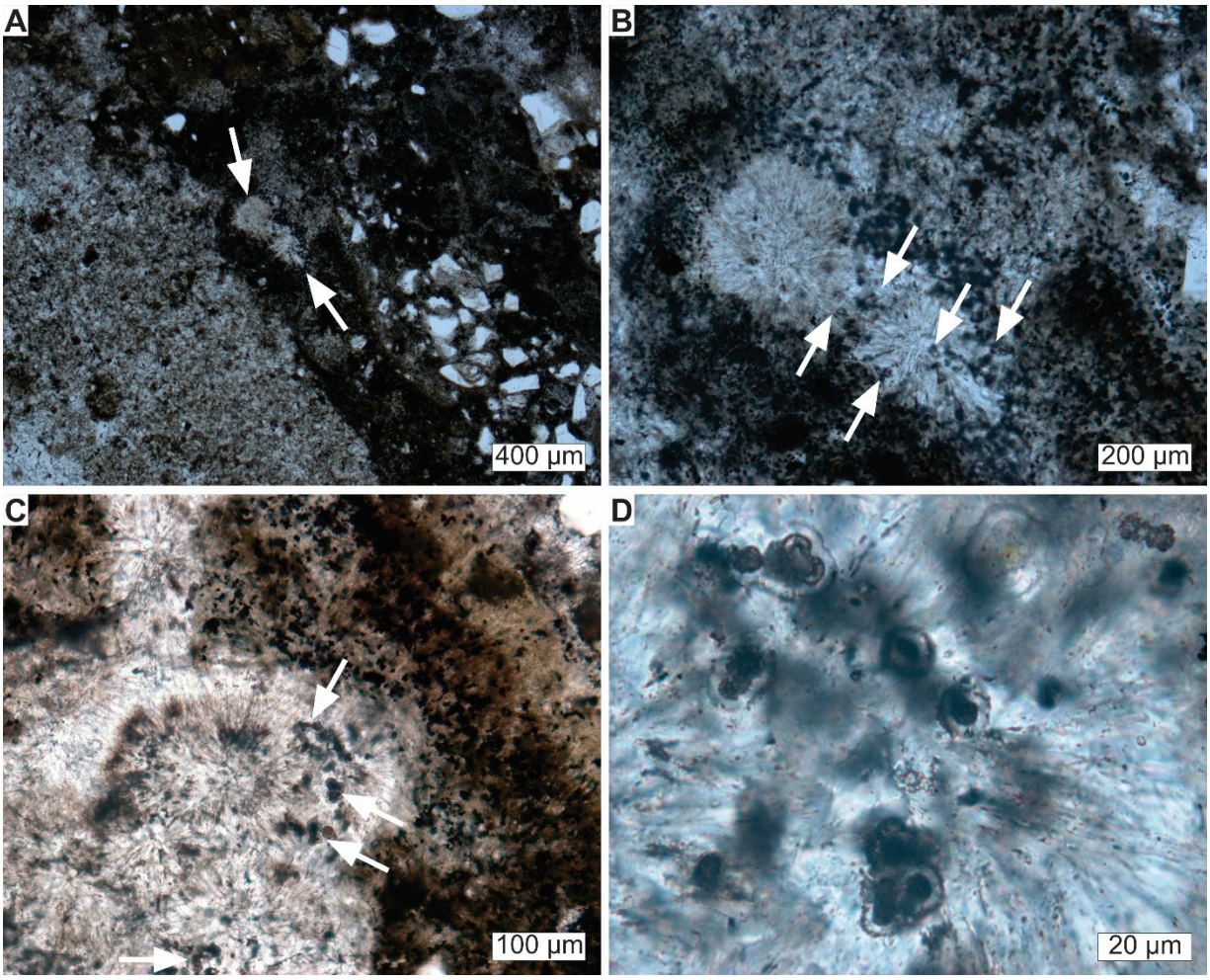

Figure S1. Thin section micrographs of microstructures (sample P1606001; parallel-polarized transmitted light). (A) Voids filled with aragonite (arrows) in inclusion-rich cement at the border to micrite-cemented sediment containing silt-sized quartz (right). (B to D) Magnified views of void-filling aragonite with microstructures (arrows); note distinct calcite layer around spheres in D.

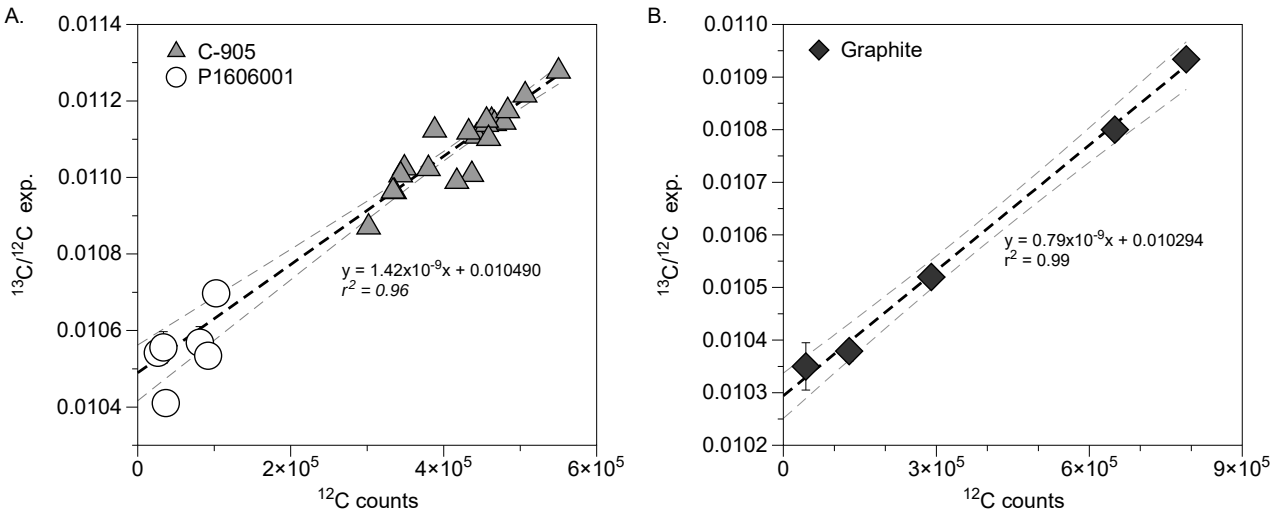

Figure S2. Correlations between experimental (exp.) $^{13}C/^{12}C$ and ^{12}C counts. (A) Standard C-905 (N = 22) and sample analyses. (B) Graphite standard; error bars are 1σ internal precision ($\sigma_{int.}$) for each analysis spot.

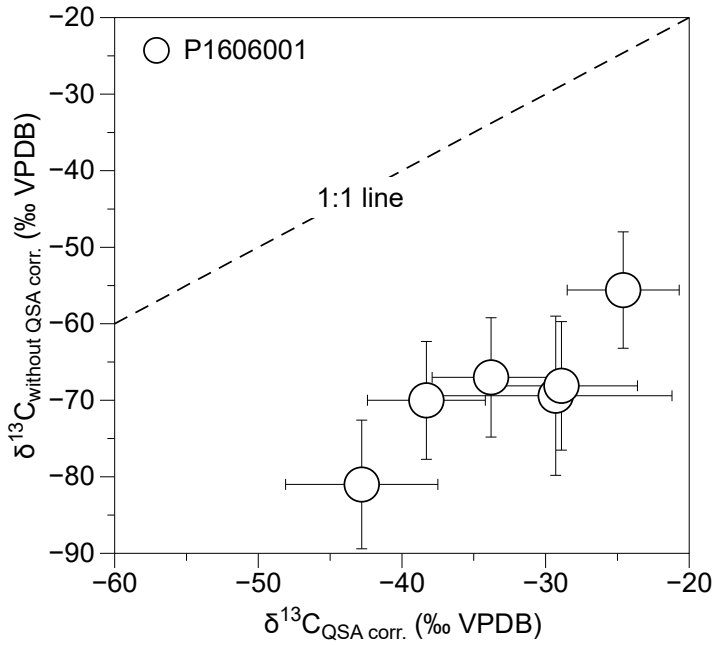

Figure S3. Relationship between sample $\delta^{13}C$ values corrected and non-corrected for QSA; error bars are total uncertainty ($\sigma_{tot.}$) calculated for each analysis spot.

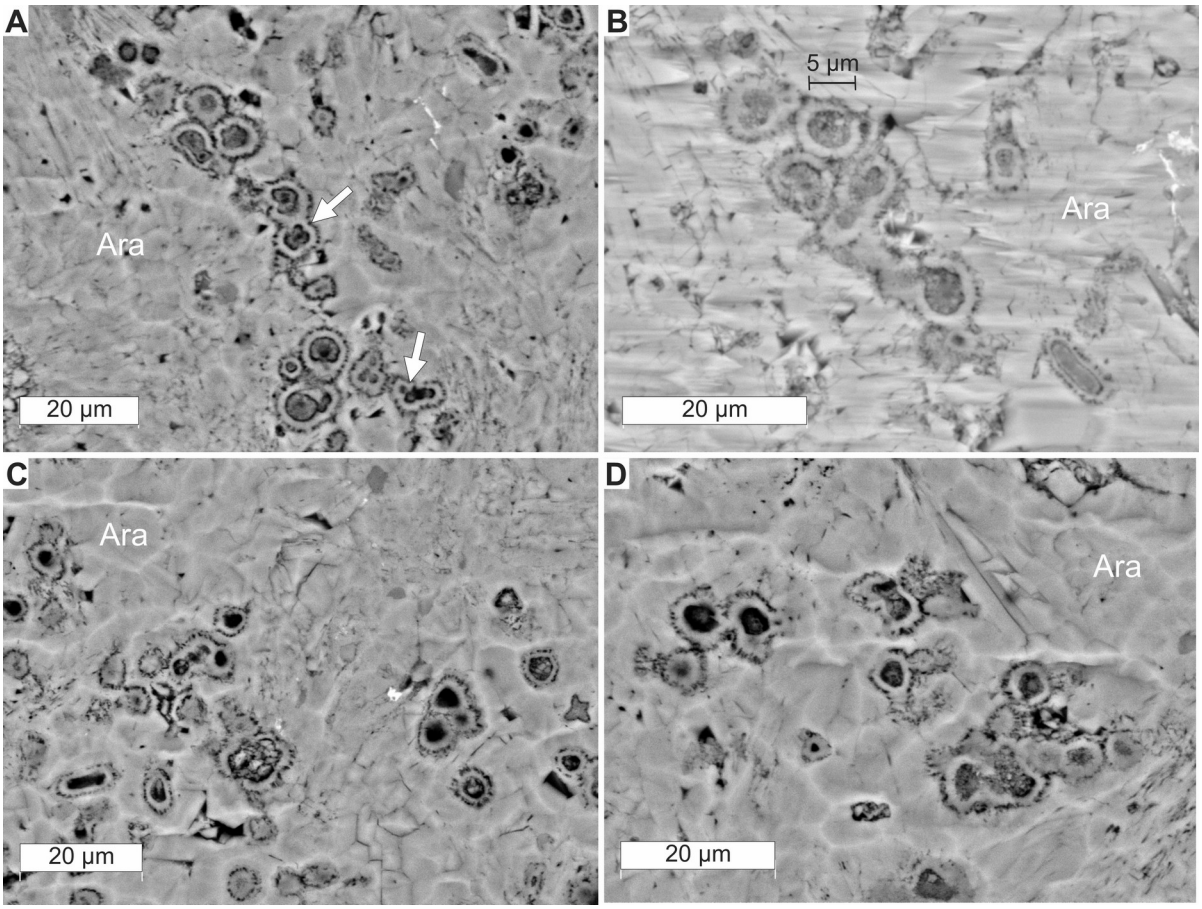

Figure S4. Thin section backscatter scanning electron microscope images showing microfacies context of clustered spheres engulfed in aragonite cement (Ara). (A) Clusters engulfed in aragonite; note areas where spheres invaginate (arrows), typical when ANME/SRB consortia divide by SRB growing into the archaeal core (Knittel et al., 2018). (B) Ion-milled surface showing cluster and size of individual sphere. (C, D) Examples of clusters and individual spheres enclosed by aragonite (Ara).

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