Supplementary Materials for

Biogenic formation of amorphous carbon by anaerobic methanotrophs and select methanogens

Kylie D. Allen, Gunter Wegener, D. Matthew Sublett Jr., Robert J. Bodnar, Xu Feng, Jenny Wendt, Robert H. White*

*Corresponding author. Email: rhwhite@vt.edu

Published 27 October 2021, Sci. Adv. 7, eabg9739 (2021)
DOI: 10.1126/sciadv.abg9739

This PDF file includes:

- Supplementary Text
- Figs. S1 to S8
- Tables S1 to S3
- References
Supplementary Text

Supplementary Methods

Analysis of amino acids and proteins in amorphous carbon samples. A 1 mg sample of the partially purified amorphous carbon (after NaOH and HCl treatments) from ANME-2a/c Seep-SRB cultures was mixed with 6 M HCl and the sample was heated under N₂ for 24 hr at 100 °C. The carbon was separated from the sample by centrifugation, the HCl was evaporated with a stream of nitrogen gas, and the resulting amino acids were dissolved in water and analyzed by TLC using solvent system [acetonitrile–water–formic acid (88%) (19:2:1 vol/vol/vol)] with ninhydrin detection. The pattern of amino acids on the TLC plate was the same as seen for the hydrolysis of ovalbumin, a typical protein. Another 1 mg of the same amorphous carbon sample was heated in 20 μl of SDS loading buffer (composed of 2.5 ml of 0.5 M tris pH 6.8, 2 ml of glycerol, 4 ml of 10% SDS, 1 ml of β-mercaptoethanol, 0.5 ml of 0.5 % bromophenol blue, and water to 20 ml) for 20 min at 100 °C. The entire sample, including the suspended carbon, was then loaded into an SDS-PAGE gel lane. After the run was complete, Coomassie staining showed only one blue band just below the bottom of the gel well. Based on the position of this band it would correspond to molecular weights of >200 kD. This band was removed, treated with trypsin and the contained peptides were analyzed using an ABSciex 4800 MALDI TOF/TOF mass spectrometer.

In another experiment, the black cell pellet from ANME-2a/c/Seep-SRB consortia was washed once with 1 M NaOH (1 ml), twice with water, followed by washing once with 1 M HCl and twice again with water. The proteins present in the NaOH and HCl wash fractions were isolated using a 3kD cutoff Amicon filter. Only the NaOH fraction contained proteins as determined by SDS-PAGE analysis and a Bradford assay. The remaining black pellet was heated with 20 μl of SDS loading buffer and centrifuged to separate out the carbon. The soluble material was loaded into one lane of an SDS-PAGE gel and the carbon pellet loaded into another lane. The purpose of this procedure was to test if any protein could be electrophoretically eluted from the isolated carbon. Except for some material that just eluted into the gel, no typical sharp protein bands were observed and instead the Coomassie stained lanes were a smear. These smears represented a multitude of different peptides resulting from proteins and degraded proteins from the biomass. Two different wide areas from this gel lane were removed and they contained many proteins identified by MALDI analysis of the tryptic peptides (major proteins identified listed in Table S2). Processing a control sample of Norit® (commercial activated carbon) did not produce any protein bands as expected.

Analysis of polycyclic aromatic hydrocarbons and elemental sulfur in the cell pellets. A 83.2 mg portion of the wet ANME-2a/c/Seep-SRB cell pellet was placed in a glass bottle along with 300 μl of methanol and 600 μl of methylene chloride. The sealed glass bottle was heated for 1-2 min at 100 °C and the resulting black residue was separated from the bulk red liquid by centrifugation. Evaporation of the solvent resulted in a solid red residue that was dissolved in methylene chloride. Half of the soluble material was applied to a TLC plate (Merck TLC silica gel 60 F254 glass plates) along with markers of pyrene and phenanthrene. The TLC plate was developed with heptane. The marker spots of pyrene and phenanthrene both eluted with a Rₜ of 0.21 and were detected by their UV absorbance and fluorescence on the TLC plate. No UV absorbing TLC bands corresponding to these compounds were detected in the sample. An intense spot with UV absorbance and a Rₜ of 0.53 was observed. This spot was scraped from the plate, eluted with hexane and in this solvent showed UV absorbance at 226, 266 and 277 nm,
the same as elemental sulfur dissolved in hexane. GC-MS analysis showed one peak with $M^+ = 255.8$ for $S_8$ that is the same as elemental sulfur. However, no polycyclic aromatic hydrocarbons were detected in this sample.

**Testing model reactions for the formation of amorphous carbon from bicarbonate.** Anaerobic 3 ml solutions containing 33 mM of each sodium bicarbonate, ferrous ammonium sulfate, and sodium hydrogen sulfide were prepared either in water or methanogenic growth media (74). The resulting samples, each containing a black suspension of iron sulfides, were heated anaerobically at 100 °C for four days and after centrifugation the clear colorless water layer was separated, and the resulting black pellet was suspended in 1 M HCl. These samples were then heated for 24 hr at 100 °C and the insoluble black material again separated by centrifugation. Heating the black pellets at 100 °C with 70% nitric acid for 1 hr dissolved all of the black pellet. This confirmed the absence of amorphous carbon, which can only be solubilized by heating for several days in 100% nitric acid.

**Supplementary Results**

**Community composition of AOM cultures.** The community composition of the two studied AOM enrichments cultures have been analyzed multiple times. The AOM50 culture consists of large consortia of ANME-1a archaea and their sulfate reducing partner bacteria of the HotSeep-1 clade (24). Metagenomic and analyses revealed that ANME and HotSeep-1 constitute about 40% and 26% of all 16SrRNA gene sequences. Methanogens are not present in this culture (24, 25, 28). According to 16S gene sequencing, the AOM20 culture contains 66% ANME 2a-c and about 10% partner bacteria of the Seep-SRB1a and Seep-SRB2 clusters. The AOM20 culture contains some methanogens that could be isolated on methylated substrates. Both cultures contain various heterotrophic minor community members that include members of the Anaerolineaceae, Calditrix and Caldisericales and archaea of the Thermoplasmatales group. These organisms likely thrive on cell exudates, supplied vitamins or AOM necromass.

**Analysis of black rubber stoppers.** Black rubber stoppers are used to seal culture bottles for the cultivation of ANME/SRB consortia. These stoppers are in contact with cell growth media for a long period of time and, thus, were tested as a possible source of the amorphous carbon observed in the cultures described here. Initial examination of the surface of the stoppers exposed to the cultures showed no sign of abrasion that would have allowed carbon to be removed from the stoppers into the media. To confirm that no carbon from the stoppers contaminated the samples, we further analyzed the stoppers by Raman spectroscopy and XPS. A portion of a black rubber stopper analyzed by Raman spectroscopy showed the presence of amorphous carbon, while the blue butyl rubber stoppers did not contain any detectible amorphous carbon, as expected. XPS analysis of new and used black rubber stoppers showed ~80% carbon and a small amount of zinc (Table S3). The high-resolution XPS C KLL spectrum showed $sp^3$ carbon in the rubber stopper (D-parameter of 14 eV, Fig. S2), while the bulk carbon in AOM cultures and methanogens is primarily $sp^2$ (D parameter of 19 eV – 21 eV, Fig. 4D, Fig. 6D, Fig. S5). Additionally, zinc was found in the rubber stopper, but zinc was not detected in any of the ANME samples. Further, the *M. maripaludis* cultures, which were shown in this work to contain amorphous carbon, were sealed with blue butyl rubber stoppers that do not contain any carbon. Thus, taken together, the data exclude the rubber stoppers from being the source of amorphous carbon we detected in cultures from organisms studied here.

**Identification of magnesium phosphate crystals observed in cell pellets from AOM consortia.** The large clear crystals clearly visible in ANME-1a/HotSeep-1 cell pellets (Fig. 1)
were identified as Mg$_3$(PO$_4$)$_2$•8H$_2$O (Bobierrite) using Raman spectroscopy (Fig. S7). These crystals were observed in both ANME-1 and ANME-2 consortia and are interpreted to have been derived from the components of the media, perhaps biochemically directed by the cells (83). Two main types of crystals observed are shown in Fig. S7A and S7B, where the first type is consistently clearer (Fig. S7A) and the second type has a “dirtier” appearance and seems to be more fibrous (Fig. S7B). The spectra of both crystals most closely resemble bobierrite (Mg$_3$(PO$_4$)$_2$•8H$_2$O) (Fig. S7), especially the fibrous crystals. The clear crystals have an additional peak at ~660 cm$^{-1}$, a more distinct peak at ~880 cm$^{-1}$, and an unidentified broad peak located at ~2500-3000 cm$^{-1}$. The spectra of the clear crystals still most closely resemble the mineral bobierrite, however it may also be produced by the related mineral baricite (MgFe)$_3$(PO$_4$)$_2$•8H$_2$O).

Tests for removal of pyrite or iron sulfides from Norit®. Control samples containing Norit® activated carbon along with pyrite (FeS$_2$) or FeS were chemically treated under similar conditions to the amorphous carbon isolated from AOM consortia and methanogens. Samples were first treated with 1 M NaOH, followed by 1 M HCl at 100 °C for 1 hr. Neither pyrite nor Norit® carbon are solubilized during this procedure. However, heating a sample of powdered FeS with HCl readily dissolved the FeS with the production of hydrogen sulfide. Additionally, heating a powdered FeS sample with a saturated solution of EDTA at 100 °C for 24 hr completely dissolves the FeS while heating a sample of FeS$_2$ under the same conditions had no effect.

The addition of 70% nitric acid (500 µl) to a suspension of Norit® (5 mg) and powdered pyrite (5 mg) at room temperature resulted in the rapid production of red NO$_2$ gas and the solubilization of the pyrite but the Norit® remained. After heating this sample for 20 min at 70 °C, the NO$_2$ gas production had ceased. After an additional 1 hr at 100 °C, the nitric acid was evaporated with a stream of nitrogen gas to leave a blackpellet. This pellet was washed three times with 1 ml of water and was dried for 24 hr at 100 °C to leave 5.8 mg of black solid. XPS elemental analysis of the sample showed C (74.6%) and O (22.5%) as the major elements and only trace amounts of N, Si and Fe, indicating that most of the pyrite in the starting mixture had been dissolved by nitric acid and the iron removed during the subsequent washings. This is consistent with the 46% weight loss observed after treatment with nitric acid.

The XPS C 1s spectra of Norit® activated carbon (Fig. S8A) shows characteristics of $sp^2$ carbon with the asymmetric peak shape centered at 284.5 eV and a satellite peak at 290.9 eV related to π-π* transition. The D parameter measured from the first derivative of C KLL spectra is 19 eV (Fig. S8B), which is close to the value of 21 eV of pure $sp^2$ carbon (graphite). The C 1s spectra of the treated sample (heated pyrite and HNO$_3$) still shows dominant $sp^2$ carbon signals (Fig. S8C) but with the addition of ether and carbonyl groups, indicating that the nitric acid treatment has functionalized a fraction of the carbon. The D parameter of 18 eV of the treated samples is slightly smaller than that of the sample before treatment (Fig. S8B and S8D), which is consistent with the reduced percentage of $sp^2$ carbon in the treated sample. These results show that treatment of Norit® samples with nitric acid has minimal effect on the chemical characteristics of the amorphous carbon.
Fig. S1. Summary of the pathways of methanogenesis and anaerobic methane oxidation. There are different methanogenic pathways depending on the substrate used — CO$_2$/H$_2$ (hydrogenotrophic methanogenesis, red pathway), methylated compounds (orange), or acetate (blue). Anaerobic methane oxidation, or reverse methanogenesis, occurs via the pathway highlighted in green. MF (methanofuran); H$_4$MPT (tetrahydromethanopterin); CoM (coenzyme M); CoA (coenzyme A).
Fig. S2. X-ray photoelectron spectra of the surface of a black rubber stopper. Based on the 14 eV signal in the C KLL spectrum, the data indicates that the carbon from the stopper is $sp^3$ as opposed to $sp^2$, thus providing evidence that excludes it as the source of the amorphous carbon detected in the cultures studied here.
Fig. S3. Spectroscopic characterization of partially purified black material containing amorphous carbon from ANME-2a/c/Seep-SRB consortia. (A) Raman spectrum, (B) C1s XPS spectrum, and (C) C KLL spectrum from the surface of the black carbon pellet. The Raman spectrum shows the G and D bands characteristic of elemental carbon materials and the XPS spectra show that the surface of the pellet is coated with protein.
Fig. S4. Mass spectra of likely iron-containing compounds identified in MALDI-MS analysis of an isolated amorphous carbon sample from ANME-1a/HotSeep-1 consortia.
MS/MS analysis of the m/z 932.3 compound showed several fragment ions, all of which contained multiple irons. These include: 873.3 (M - 59.0), 859.3 (M - 73.0), 802.3 (M - 130.0), and 743.3 (M -189.0). MS/MS analysis of the m/z 1329.4 compound showed intense fragment ions at 1183.4 (M -147.0), 1051.4 (M -279.0), 989.4 (M -340.0), and 971.4 (M -358.0), again each fragment ion contained multiple irons based upon the M-2 isotope peak due to $^{54}\text{Fe}$. 
Fig. S5. X-ray photoelectron spectra (XPS) of the amorphous carbon isolated from three different methanogenic archaea. The spectra corresponding to the bulk carbon from *M. maripaludis* are also shown in Fig. 6 of the main text. The first column contains the C 1s spectra and the second column contains the first derivative C KLL spectra. The surface of all samples contains proteins/peptides, and thus minimal information can be obtained about the characteristics of the associated amorphous carbon. After etching of the samples to access the bulk carbon in the sample (below the surface of the pellet), the C 1s spectra reveal primarily C-C/C=C bonds and the C KLL first derivative spectra show that the carbon is primarily $sp^2$. These XPS data show that the carbon isolated from the different methanogens has similar characteristics to the carbon isolated from AOM cultures. MMP: *Methanococcus maripaludis*; MJ: *Methanocaldococcus jannaschi*; MB: *Methanosarcina barkeri*. 
S-\(5'\)-adenosyl-L-methionine
(SAM)

\[\text{H}_2\text{N} \quad \text{COOH}\]

1-aminocyclopropane-1-carboxylic acid
(ACC)

\[\text{H}_2\text{C} = \text{CH}_2\]

\[\text{H}_2\]

\[\text{CO}_2 \text{ or CH}_4\]

\[?\]

\[\text{resonance-stabilized radical species}\]

polycyclic aromatic hydrocarbons

\[\text{(HC} = \text{CH})_n\]

“Soot particles”

**Fig. S6. A model for the radical formation of soot carbon.** Ethylene and/or acetylene radicals undergo radical chain reactions to generate polycyclic aromatic hydrocarbons that are further condensed to form the final soot particles.
Fig. S7. Photomicrographs of the two types of crystals (A, B) observed in ANME-2a/c/Seep-SRB cultures and analyzed by Raman spectroscopy (right panel). Spectra obtained from the two crystals are shown with reference spectra of the two most likely candidates for the identity of the crystal, boberrite ($\text{Mg}_3(\text{PO}_4)_2\cdot 8\text{H}_2\text{O}$) and baricite ($\text{MgFe}_3(\text{PO}_4)_2\cdot 8\text{H}_2\text{O}$).
Fig. S8. C 1s and C KLL XPS spectra of Norit® (commercial activated carbon) and pyrite mixture after nitric acid treatments. This experiment was performed to show that the chemical characteristics of the carbon do not change significantly after treatment with strong acid in the presence of pyrite. This suggests that the analyses performed on the amorphous carbon isolated from the organisms studied here reveals the true nature of the biochemically formed amorphous carbon.
Table S1. Metabolic and isotope data from stable isotope incubations with the AOM50 culture. AC= amorphous carbon, TBC= total biomass carbon (includes the inorganic carbon).

| Experiment | Incubation time (days) | Sulfide formation (mM) | $\delta^{13}$CH$_4$ ($T_0$) | $\delta^{13}$C-DIC ($T_0$) | $\delta^{13}$C-TBC ($T_0$) | $\delta^{13}$C-AC formation ($T_0$) | TBC formation ($\%$ of TBC) | AC formation ($\%$ of AC) |
|------------|------------------------|------------------------|-----------------------------|---------------------------|--------------------------|-------------------------------|-----------------------------|--------------------------|
| +CH$_4$+${}^{13}$C-DIC | 5 | 1.82 | -42 | -2 | 5083 | 3692 | 234 | 251 | 7.16 | 31 | 1.87 |
| +CH$_4$+${}^{13}$C-DIC | 11 | 3.42 | -42 | 32 | 4996 | 4118 | 397 | 381 | 10.22 | 38 | 1.98 |
| +CH$_4$+${}^{13}$C-DIC | 20 | 7.13 | -41 | 109 | 4933 | 3575 | 577 | 581 | 15.45 | 173 | 5.38 |
| +CH$_4$+${}^{13}$C-DIC | 20 | 7.24 | -41 | 117 | 4930 | 3582 | 562 | 631 | 15.87 | 209 | 6.25 |
| +${}^{13}$CH$_4$ | 5 | 1.85 | 7370 | 7299 | 7 | 571 | -8 | -2 | 0.83 | -26 | 0.33 |
| +${}^{13}$CH$_4$ | 11 | 4.25 | 6910 | 6772 | 6 | 967 | 52 | 53 | 1.78 | -11 | 0.59 |
| +${}^{13}$CH$_4$ | 20 | 7.64 | 6965 | 6705 | 12 | 1594 | 176 | 160 | 3.59 | 25 | 1.15 |
| +${}^{13}$C-DIC | 5 | 0.04 | - | - | 5260 | 4522 | -54 | -60 | 0.11 | -39 | 0.19 |
| +${}^{13}$C-DIC | 11 | 0.33 | - | - | 5117 | n.m. | -54 | -58 | 0.13 | -42 | 0.14 |
| +${}^{13}$C-DIC | 20 | 0.15 | - | - | 4997 | 4368 | -52 | -55 | 0.18 | -41 | 0.17 |
| T$\_0$ sample | - | - | - | - | - | -61 | -63 | -48 |
Table S2. The top 20 hits for proteins identified by MALDI-MS on the surface of a black carbon pellet isolated from ANME-2a/c/Seep-SRB cultures.

| Protein hit number | Protein accession number | Protein description                                                                 |
|--------------------|--------------------------|------------------------------------------------------------------------------------|
| 1                  | A0A2P5K3A1               | Methyl-coenzyme M reductase subunit gamma OS=ANME-2 cluster archaeon HR1 OX=1968520 GN=mcrG |
| 1                  | A0A062V5Q9               | Methyl-coenzyme M reductase subunit gamma OS=Candidatus Methanoperedens nitroreducens OX=1392998 GN=ANME2D_01103 |
| 2                  | A0A2H4Y9E9               | Methyl-coenzyme M reductase alpha subunit (Fragment) OS=uncultured euryarchaeote OX=114243 GN=mcrA |
| 3                  | A0A368P960               | Methyl-coenzyme M reductase subunit beta OS=ANME-2 cluster archaeon OX=2056317 GN=mcrB |
| 4                  | A0A127AWV7               | Adenylylsulfate reductase alpha subunit (Fragment) OS=uncultured microorganism OX=358574 GN=aprA |
| 5                  | A0A2P5K1X0               | 5,10-methylenetetrahydromethanopterin reductase OS=ANME-2 cluster archaeon HR1 OX=1968520 GN=mer |
| 6                  | A0A2U0RW15               | Uncharacterized protein OS=Candidatus Bathyarchaeota archaeon OX=2026714 GN=CW691_10990 |
| 7                  | A0A2P5K3A0               | Methyl-coenzyme M reductase subunit beta OS=ANME-2 cluster archaeon HR1 OX=1968520 GN=mcrB |
| 8                  | H8YUX9                   | DsrA (Fragment) OS=uncultured archaeon OX=115547 GN=dsrA |
| 9                  | A0A2N2H3A5               | F0F1 ATP synthase subunit beta (Fragment) OS=Deltaproteobacteria bacterium HGW-Deltaproteobacteria-21 OX=2013749 GN=atpD |
| 10                 | A0A523R0Y9               | ATP synthase subunit alpha OS=Desulfobacteraceae bacterium OX=2049433 GN=atpA |
| 11                 | A0A127AWZ6               | Dissimilatory sulfite reductase alpha subunit (Fragment) OS=uncultured microorganism OX=358574 GN=dsrA |
| 12                 | A0A0P8A173               | Methyl-coenzyme M reductase subunit beta OS=Candidatus Methanoperedens sp. BLZ1 OX=1719120 GN=MPEBLZ_01204 |
| 13                 | A0A062V9E2               | Membrane protease subunit, stomatin/prohibitin OS=Candidatus Methanoperedens nitroreducens OX=1392998 GN=ANME2D_00801 |
| 14                 | Q2VP82                   | CoB--CoM heterodisulfide reductase iron-sulfur subunit A OS=uncultured archaeon OX=115547 GN=C1_0004 |
|   | Accession  | Description                                                                 | Organism                      | Accession | Gene Name       |
|---|------------|------------------------------------------------------------------------------|-------------------------------|-----------|-----------------|
| 15| A0A2P5K613 | Elongation factor 1-alpha OS=ANME-2 cluster archaeon HR1 OX=1968520 GN=tuf  |                                |           |                 |
| 16| A0A533MKE5 | TIGR00266 family protein OS=ANME-2 cluster archaeon OX=2056317 GN=C5S46_05885 |                                |           |                 |
| 17| A0A497M2X6 | Chaperonin GroEL OS=Candidatus Bathyarchaeota archaeon OX=2026714 GN=groL    |                                |           |                 |
| 18| A0A524PYP4 | NADH-quinone oxidoreductase subunit I OS=ANME-2 cluster archaeon OX=2056317 GN=E4G94_08895 |                                |           |                 |
| 19| A0A0F9IF81 | AdoHcyase_NAD domain-containing protein (Fragment) OS=marine sediment metagenome OX=412755 GN=LCGC14_1586520 |                                |           |                 |
| 20| A0A0P7ZDJ4 | Uncharacterized protein OS=Candidatus Methanoperedens sp. BLZ1 OX=1719120 GN=MPEBLZ_02702 |                                |           |                 |
Table S3. Surface elemental composition derived from X-ray photoelectron spectroscopy analysis of rubber stoppers and Norit® (activated carbon). Data reported as atom%.

| Sample                        | C   | N   | O   | F   | Na  | Mg  | Al  | Si  | P   | S   | Cl  | Ca  | Fe  | Co  | Zn  | Br  |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Black rubber stopper new      | 80.1| -   | 12.3| 2.1 | -   | -   | -   | 3.8 | -   | 0.5 | -   | 0.1 | -   | -   | 0.7 | 0.3 |
| Black rubber stopper used     | 81.9| -   | 11.4| -   | 0.2 | 0.6 | -   | 3.4 | -   | 0.4 | 1.5 | 0.3 | -   | -   | 0.3 | -   |
| Activated carbon              | 93.3| -   | 6.5 | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
REFERENCES AND NOTES

1. D. D. L. Chung, Review graphite. *J. Mater. Sci.* **37**, 1475–1489 (2002).
2. A. Petzold, J. A. Ogren, M. Fiebig, P. Laj, S. M. Li, U. Baltensperger, T. Holzer-Popp, S. Kinne, G. Pappalardo, N. Sugimoto, C. Wehrli, A. Wiedensohler, X. Y. Zhang, Recommendations for reporting “black carbon” measurements. *Atmos. Chem. Phys.* **13**, 8365–8379 (2013).
3. N. L. Briggs, C. M. Long, Critical review of black carbon and elemental carbon source apportionment in Europe and the United States. *Atmos. Environ.* **144**, 409–427 (2016).
4. Q. H. S. Chan, M. E. Zolensky, R. J. Bodnar, C. Farley, J. C. H. Cheung, Investigation of organo-carbonate associations in carbonaceous chondrites by Raman spectroscopy. *Geochim. Cosmochim. Acta* **201**, 392–409 (2017).
5. N. Singh, S. Abiven, M. S. Torn, M. W. I. Schmidt, Fire-derived organic carbon in soil turns over on a centennial scale. *Biogeosciences* **9**, 2847–2857 (2012).
6. S. Bruun, E. S. Jensen, L. S. Jensen, Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Org. Geochem.* **39**, 839–845 (2008).
7. M. C. Potter, Bacteria as agents in the oxidation of amorphous carbon. *Proc. R. Soc. Lond. B-Conta* **80**, 239–259 (1908).
8. Y. Kuzyakov, I. Subbotina, H. Q. Chen, I. Bogomolova, X. L. Xu, Black carbon decomposition and incorporation into soil microbial biomass estimated by $^{14}$C labeling. *Soil Biol. Biochem.* **41**, 210–219 (2009).
9. A. R. Zimmerman, Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environ. Sci. Technol.* **44**, 1295–1301 (2010).
10. M. Szczesna-Antczak, A. Kaczmorska, W. Kaczmorski, T. Antczak, Biomodification and biodeterioration of carbon coatings by fungal strains. *Int. Biodeter. Biodegr.* **88**, 106–117 (2014).
11. L. M. Sekhohola, M. L. Isaacs, A. K. Cowan, Fungal colonization and enzyme-mediated metabolism of waste coal by Neosartorya fischeri strain ECCN 84. *Biosci. Biotechnol. Biochem.* **78**, 1797–1802 (2014).
12. Z. Lyu, N. Shao, T. Akinyemi, W. B. Whitman, Methanogenesis. *Curr. Biol.* **28**, R727-R732 (2018).
13. S. Kirschke, P. Bousquet, P. Clais, M. Saunois, J. G. Canadell, E. J. Dlugokencky, P. Bergamaschi, D. Bergmann, D. R. Blake, L. Bruhwiler, P. Cameron-Smith, S. Castaldi, F. Chevallier, L. Feng, A. Fraser, M. Heimann, E. L. Hodson, S. Houweling, B. Josse, P. J. Fraser, P. B. Krummel, J. F. Lamarque, R. L. Langenfelds, C. le Quéré, V. Naik, S. O’Doherty, P. I. Palmer, I. Pison, D.
Plummer, B. Poulter, R. G. Prinn, M. Rigby, B. Ringleval, M. Santini, M. Schmidt, D. T. Shindell, I. J. Simpson, R. Spahni, L. P. Steele, S. A. Strode, K. Sudo, S. Szopa, G. R. van der Werf, A. Voulgarakis, M. van Weele, R. F. Weiss, J. E. Williams, G. Zeng, Three decades of global methane sources and sinks. *Nat. Geosci.* 6, 813–823 (2013).

14. S. J. Hallam, N. Putnam, C. M. Preston, J. C. Dettter, D. Rokhsar, P. M. Richardson, E. DeLong, Reverse methanogenesis: Testing the hypothesis with environmental genomics. *Science* 305, 1457–1462 (2004).

15. S. Bhattarai, C. Cassarini, P. N. L. Lens, Physiology and distribution of archaeal methanotrophs that couple anaerobic oxidation of methane with sulfate reduction. *Microbiol. Mol. Biol. Rev.* 83, 10.1128/MMBR.00074-18 (2019).

16. W. S. Reeburgh, Oceanic methane biogeochemistry. *Chem. Rev.* 107, 486–513 (2007).

17. K. U. Hinrichs, J. M. Hayes, S. P. Sylva, P. G. Brewer, E. F. DeLong, Methane-consuming archaeabacteria in marine sediments. *Nature* 398, 802–805 (1999).

18. V. J. Orphan, K. U. Hinrichs, W. Ussler III, C. K. Paull, L. T. Taylor, S. P. Sylva, J. M. Hayes, E. F. Delong, Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Appl. Environ. Microbiol.* 67, 1922–1934 (2001).

19. A. A. Raghoebarsing, A. Pol, K. T. van de Pas-Schoonen, A. J. P. Smolders, K. F. Ettwig, W. I. C. Rijpstra, S. Schouten, J. S. S. Damsté, H. J. M. op den Camp, M. S. M. Jetten, M. Strous, A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440, 918–921 (2006).

20. M. F. Haroon, S. Hu, Y. Shi, M. Imelfort, J. Keller, P. Hugenholtz, Z. Yuan, G. W. Tyson, Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* 500, 567–570 (2013).

21. K. Knittel, T. Losekann, A. Boetius, R. Kort, R. Amann, Diversity and distribution of methanotrophic archaea at cold seeps. *Appl. Environ. Microbiol.* 71, 467–479 (2005).

22. P. H. Timmers, C. U. Welte, J. J. Koehorst, C. M. Plugge, M. S. M. Jetten, A. J. M. Stams, Reverse methanogenesis and respiration in methanotrophic archaea. *Archaeb* 2017, 1654237 (2017).

23. S. E. McGlynn, G. L. Chadwick, C. P. Kempe, V. J. Orphan, Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526, 531–535 (2015).

24. G. Wegener, V. Krukenberg, D. Riedel, H. E. Tegetmeyer, A. Boetius, Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature* 526, 587–590 (2015).
25. V. Krukenberg, D. Riedel, H. R. Gruber-Vodicka, P. L. Buttigieg, H. E. Tegetmeyer, A. Boetius, G. Wegener, Gene expression and ultrastructure of meso- and thermophilic methanotrophic consortia. *Environ. Microbiol.* **20**, 1651–1666 (2018).

26. A. Boetius, K. Ravensschlag, C. J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B. B. Jørgensen, U. Witte, O. Pfannkuche, A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**, 623–626 (2000).

27. T. Holler, F. Widdel, K. Knittel, R. Amann, M. Y. Kellermann, K.U. Hinrichs, A. Teske, A. Boetius, G. Wegener, Thermophilic anaerobic oxidation of methane by marine microbial consortia. *ISME J.* **5**, 1946–1956 (2011).

28. G. Wegener, V. Krukenberg, S. E. Ruff, M. Y. Kellermann, K. Knittel, Metabolic capabilities of microorganisms involved in and associated with the anaerobic oxidation of methane. *Front. Microbiol.* **7**, (2016).

29. T. Holler, G. Wegener, K. Knittel, A. Boetius, B. Brunner, M. M. M. Kuypers, F. Widdel, Substantial (13) C/(12) C and D/H fractionation during anaerobic oxidation of methane by marine consortia enriched in vitro. *Environ. Microbiol. Rep.* **1**, 370–376 (2009).

30. V. Krukenberg, K. Harding, M. Richter, F. O. Glöckner, H. R. Gruber-Vodicka, B. Adam, J. S. Berg, K. Knittel, H. E. Tegetmeyer, A. Boetius, G. Wegener, Candidatus Desulfofervidus auxilii, a hydrogenotrophic sulfate-reducing bacterium involved in the thermophilic anaerobic oxidation of methane. *Environ. Microbiol.* **18**, 3073–3091 (2016).

31. R. Burgess, C. Buono, P. R. Davies, R. J. Davies, T. Legge, A. Lai, R. Lewis, D. J. Morgan, N. Robinson, D. J. Willock, The functionalisation of graphite surfaces with nitric acid: Identification of functional groups and their effects on gold deposition. *J. Catal.* **323**, 10–18 (2015).

32. A. C. Ferrari, Raman spectroscopy of graphene and graphite: Disorder, electron-phonon coupling, doping and nonadiabatic effects. *Solid State Commun.* **143**, 47–57 (2007).

33. A. Merlen, J. G. Buijnsters, C. Pardanaud, A guide to and review of the use of multiwavelength raman spectroscopy for characterizing defective aromatic carbon solids: From graphene to amorphous carbons. *Coatings* **7**, 153 (2017).

34. A. C. Ferrari, J. Robertson, Interpretation of Raman spectra of disordered and amorphous carbon. *Phys. Rev. B* **61**, 14095–14107 (2000).

35. W. J. B. Dufresne, C. J. Rufledt, C. P. Marshall, Raman spectroscopy of the eight natural carbonate minerals of calcite structure. *J. Raman Spectrosc.* **49**, 1999–2007 (2018).
36. J. Diaz, G. Paolicelli, S. Ferrer, F. Comin, Separation of the sp3 and sp2 components in the C1s photoemission spectra of amorphous carbon films. *Phys Rev B* **54**, 8064–8069 (1996).
37. A. Mezzi, S. Kaciulis, Surface investigation of carbon films: From diamond to graphite. *Surf. Interface Anal.* **42**, 1082–1084 (2010).
38. H. Torabizadeh, All proteins have a basic molecular formula. *World Acad. Sci. Eng. Technol.* **78**, 961–965 (2011).
39. V. J. Orphan, C. H. House, K. U. Hinrichs, K. D. McKeegan, E. F. DeLong, Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* **293**, 484–487 (2001).
40. M. Y. Kellermann, G. Wegener, M. Elvert, M. Y. Yoshinaga, Y.S. Lin, T. Holler, X. P. Mollar, K. Knittel, K.U. Hinrichs, Autotrophy as a predominant mode of carbon fixation in anaerobic methane-oxidizing microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 19321–19326 (2012).
41. T. Treude, V. Orphan, K. Knittel, A. Gieseke, C. H. House, A. Boetius, Consumption of methane and CO2 by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. *Appl. Environ. Microbiol.* **73**, 2271–2283 (2007).
42. M. V. Kharlamova, V. N. Mochalin, M. R. Lukatskaya, J. Niu, V. Presser, S. Mikhalovsky, Y. Gogotsi, Adsorption of proteins in channels of carbon nanotubes: Effect of surface chemistry. *Mater. Express* **3**, 1–10 (2013).
43. S. Ray, A. G. Shard, Quantitative analysis of adsorbed proteins by x-ray photoelectron spectroscopy. *Anal. Chem.* **83**, 8659–8666 (2011).
44. A. Cuesta, P. Dhamelincourt, J. Laureyns, A. Martinezalonso, J. M. D. Tascon, Raman microprobe studies on carbon materials. *Carbon* **32**, 1523–1532 (1994).
45. N. K. Lunsdorf, I. Dunkl, B. C. Schmidt, G. Rantitsch, H. von Eynatten, Towards a higher comparability of geothermometric data obtained by raman spectroscopy of carbonaceous material. Part I: Evaluation of biasing factors. *Geostand. Geoanal. Res.* **38**, 73–94 (2014).
46. M. J. Whiticar, Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.* **161**, 291–314 (1999).
47. H. Niemann, M. Elvert, Diagnostic lipid biomarker and stable carbon isotope signatures of microbial communities mediating the anaerobic oxidation of methane with sulphate. *Org. Geochem.* **39**, 1668–1677 (2008).
48. E. A. Mathez, J. R. Delaney, The nature and distribution of carbon in submarine basalts and peridotite nodules. *Earth Planet. Sci. Lett.* **56**, 217–232 (1981).

49. G. D. Renshaw, C. Roscoe, P. L. Walker, Disproportionation of CO: 1. Over iron and silicon-iron single crystals. *J. Catal.* **18**, 164-183 (1970).

50. J. Jedwab, J. Boulegue, Graphite crystals in hydrothermal vents. *Nature* **310**, 41–43 (1984).

51. V. Matjuschkin, A. B. Woodland, D. J. Frost, G. M. Yaxley, Reduced methane-bearing fluids as a source for diamond. *Sci. Rep.* **10**, 6961 (2020).

52. K. O. Johansson, M. P. Head-Gordon, P. E. Schrader, K. R. Wilson, H. A. Michelsen, Resonance-stabilized hydrocarbon-radical chain reactions may explain soot inception and growth. *Science* **361**, 997–1000 (2018).

53. M. Frenklach, Reaction mechanism of soot formation in flames. *Phys. Chem. Chem. Phys.* **4**, 2028–2037 (2011).

54. H. Wang, Formation of nascent soot and other condensed-phase materials in flames. *Proc. Combust Inst.* **33**, 41–67 (2011).

55. D. O. Adams, S. F. Yang, 1-aminocyclopropane-1-carboxylate synthase. *Methods Enzymol.* **143**, 426–429 (1987).

56. B. Schink, Inhibition of methanogenesis by ethylene and other unsaturated-hydrocarbons. *FEMS Microbiol. Ecol.* **31**, 63–68 (1985).

57. R. K. Thauer, Biochemistry of methanogenesis: A tribute to Marjory Stephenson. 1998 Marjory Stephenson Prize Lecture. *Microbiology* **144** (Pt 9), 2377–2406 (1998).

58. A. E. Rotaru, P. M. Shrestha, F. Liu, B. Markovaite, S. Chen, K. P. Nevin, D. R. Lovley, Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. *Appl. Environ. Microbiol.* **80**, 4599–4605 (2014).

59. A. E. Rotaru, P. M. Shrestha, F. Liu, M. Shrestha, D. Shrestha, M. Embree, K. Zengler, C. Wardman, K. P. Nevin, D. R. Lovley, A new model for electron flow during anaerobic digestion: Direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. *Energ. Environ. Sci.* **7**, 408–415 (2014).

60. S. Scheller, H. Yu, G. L. Chadwick, S. E. McGlynn, V. J. Orphan, Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. *Science* **351**, 703–707 (2016).

61. C. T. Skennerton, K. Chourey, R. Iyer, R. L. Hettich, G. W. Tyson, V. J. Orphan, Erratum for Skennerton et al., “methane-fueled syntrophy through extracellular electron transfer: Uncovering the
genomic traits conserved within diverse bacterial partners of anaerobic methanotrophic archaea”. *MBio* **8**, 10.1128/mBio.01561-17 (2017).

62. D. D. L. Chung, Electrical applications of carbon materials. *J. Mater. Sci.* **39**, 2645–2661 (2004).

63. L. Kavan, Electrochemical carbon. *Chem. Rev.* **97**, 3061–3082 (1997).

64. F. H. Liu, A.-E. Rotaru, P. M. Shrestha, N. S. Malvankar, K. P. Nevin, D. R. Lovley, Promoting direct interspecies electron transfer with activated carbon. *Energ. Environ. Sci.* **5**, 8982–8989 (2012).

65. P. R. Yaashikaa, P. S. Kumar, S. Varjani, A. Saravanan, A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy. *Biotechnol. Rep. (Amst.)* **28**, e00570 (2020).

66. S. Chen, A.-E. Rotaru, P. M. Shrestha, N. S. Malvankar, F. Liu, W. Fan, K. P. Nevin, D. R. Lovley, Promoting interspecies electron transfer with biochar. *Sci. Rep.* **4**, 5019 (2014).

67. J. Ma, J. Pan, L. Qiu, Q. Wang, Z. Zhang, Biochar triggering multipath methanogenesis and subdued propionic acid accumulation during semi-continuous anaerobic digestion. *Bioresour. Technol.* **293**, 122026 (2019).

68. J. M. Saquing, Y. H. Yu, P. C. Chiu, Wood-derived black carbon (biochar) as a microbial electron donor and acceptor. *Environ. Sci. Technol. Lett.* **3**, 62–66 (2016).

69. L. Klupfel, M. Keiluweit, M. Kleber, M. Sander, Redox properties of plant biomass-derived black carbon (biochar). *Environ. Sci. Technol.* **48**, 5601–5611 (2014).

70. X. Q. Zhang, J. Xia, J. Pu, C. Cai, G. W. Tyson, Z. Yuan, S. Hu, Biochar-mediated anaerobic oxidation of methane. *Environ. Sci. Technol.* **53**, 6660–6668 (2019).

71. S. Kar, S. K. Mandal, D. Das, S. Chaudhuri, Wet chemical synthesis of iron pyrite and characterization by Mössbauer spectroscopy. *Mater. Lett.* **58**, 2886–2889 (2004).

72. F. B. Widdel, *The Prokaryotes*, D. M. Trüper, W. Harder, K.-H. Schleifer, Eds. (Springer, New York, ed. 2, 2002).

73. R. Laso-Perez, V. Krukenberg, F. Musat, G. Wegener, Establishing anaerobic hydrocarbon-degrading enrichment cultures of microorganisms under strictly anoxic conditions. *Nat. Protoc.* **13**, 1310–1330 (2018).

74. F. Sarmiento, J. A. Leigh, W. B. Whitman, Genetic systems for hydrogenotrophic methanogens. *Methods Enzymol.* **494**, 43–73 (2011).

75. F. Long, L. L. Wang, B. Lupa, W. B. Whitman, A flexible system for cultivation of *methanococcus* and other formate-utilizing methanogens. *Archaea* **2017**, 1-12 (2017).
76. K. R. Sowers, J. E. Boone, R. P. Gunsalus, Disaggregation of methanosarcina spp. and growth as single cells at elevated osmolarity. *Appl. Environ. Microbiol.* **59**, 3832–3839 (1993).

77. B. Mukhopadhyay, E. F. Johnson, R. S. Wolfe, Reactor-scale cultivation of the hyperthermophilic methanarchaeon *Methanococcus jannaschii* to high cell densities. *Appl. Environ. Microbiol.* **65**, 5059–5065 (1999).

78. R. H. White, Identification and biosynthesis of 1-mercaptoethanesulfonic acid (1-MES), an analogue of coenzyme M, found widely in the methanogenic archaea. *Biochemistry* **56**, 6137–6144 (2017).

79. F. C. Neidhardt, H. E. Umbarger, Chemical Composition of *Escherichia coli* in, *Escherichia coli and Salmonella Cellular and Molecular Biology*, F. C. Neidhardt, Ed. (ASM Press, Washington D.C., ed. 2, 1996), vol. 1.

80. A. C. Allwood, M. R. Walter, C. P. Marshall, Raman spectroscopy reveals thermal palaeoenvironments of c.3.5 billion-year-old organic matter. *Vib. Spectrosc.* **41**, 190–197 (2006).

81. D. G. Henry, I. Jarvis, G. Gillmore, M. Stephenson, J. F. Emmings, Assessing low-maturity organic matter in shales using Raman spectroscopy: Effects of sample preparation and operating procedure. *Int. J. Coal Geol.* **191**, 135–151 (2018).

82. O. Beyssac, B. Goffé, J. P. Petitet, E. Froigneux, M. Moreau, J. N. Rouzaud, On the characterization of disordered and heterogeneous carbonaceous materials by Raman spectroscopy. *Spectrochim. Acta A* **59**, 2267–2276 (2003).

83. G. M. Gadd, Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiol. Sgm.* **156**, 609–643 (2010).