Table S1. Temporal development of carbon isotopic compositions of bacterial fatty acids (in ‰) compared to DIC in AOM enrichments from the Guaymas Basin at 37°C and 50°C (AOM37 and 50, respectively) over 28 days and the cultured bacterial partner Ca. D. auxilii over 40 days using different incubation conditions. n.a.: not available due to low signal during isotope analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Experiment: condition | Time (days) | DIC | C14 | *i*C15 | *ai*C15 | *i*C16 | C16:17 | C16 | *i*C17 | *ai*C17 | C17:16 | C17 | C18:19 | C18:17 | C18 |
| AOM37 | 1: + CH4 + 13C-leu | 0 | -15 | -24 | -49 | -46 | -51 | -55 | -38 | -59 | -61 | -68 | n.a | -19 | -63 | -30 |
|  |  | 0.5 | -14 | -27 | 97 | 230 | 58 | -46 | -33 | 260 | 47 | -53 | -51 | 48 | -52 | -30 |
|  |  | 3 | -10 | -18 | 600 | 950 | 230 | -28 | -27 | 810 | 160 | -16 | 61 | 320 | -10 | -28 |
|  | 7 | 1 | -10 | 930 | 1300 | 300 | -8 | -19 | 1300 | 250 | 4 | 250 | 760 | 50 | -22 |
|  | 14 | 18 | 3 | 1700 | 2100 | 530 | -2 | -11 | 1800 | 340 | 18 | 430 | 1600 | 98 | -15 |
|  | 28 | 51 | 22 | 2300 | 2800 | 730 | 31 | -1 | 2300 | 400 | 51 | 700 | 2200 | 120 | -12 |
| 2: + CH4 | 28 | -20 | -25 | -50 | -46 | -52 | -55 | -38 | -59 | -61 | -68 | n.a | -17 | -63 | -31 |
| 3: + 13C-leu | 28 | 68 | 24 | 1600 | 2800 | 840 | -88 | -17 | 1800 | 330 | n.a | 410 | 2300 | 82 | -13 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| AOM50 | 1: + CH4 + 13C-leu | 0 | -26 | -25 | n.a | -26 | -45 | -27 | -28 | n.a | -45 | n.a | -27 | -25 | -35 | -28 |
|  |  | 28 | 113 | 21 | 6400 | 1300 | 590 | 180 | 5 | 3900 | -21 | 51 | 470 | 130 | 110 | -20 |
| 2: + CH4 | 28 | -34 | -25 | n.a | -26 | -45 | -27 | -28 | n.a | -46 | n.a | -27 | -25 | -36 | -28 |
| 3: + 13C-leu | 28 | 125 | -19 | 5700 | 970 | n.a | -26 | -23 | 3700 | 170 | n.a | 230 | 14 | n.a | -29 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Ca.* D. auxilii | 4: + H2 + 13C-leu | 0 | -18 | -25 | n.a. | n.a. | n.a | -28 | -24 | n.a. | n.a. | n.a | -23 | n.a. | -27 | -22 |
|  | 40 | -18 | -26 | n.a. | n.a. | n.a | n.a | -26 | n.a. | n.a. | n.a | -24 | n.a. | -28 | -24 |

Table S2. Temporal development of carbon isotopic compositions of archaeal lipids (in ‰), separated into core and intact polar lipids (CL and IPL, respectively), compared to DIC in AOM enrichments from the Guaymas Basin at 37°C and 50°C (AOM37 and 50, respectively) over 28 days using different incubation conditions. n.a.: not available due to low signal during isotope analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Experiment: condition | Time (days) | DIC | CL-Phy | CL-BP0 | CL-BP1 | CL-BP2 | IPL-Phy | IPL-BP0 | IPL-BP1 | IPL-BP2 |
| AOM37 | 1: + CH4 + 13C-leu | 0 | -15 | -72 | -71 | -73 | -72 | -71 | -70 | -71 | -68 |
|  | 0.5 | -14 | -71 | -71 | -72 | -71 | -60 | n.a | n.a | n.a |
|  | 3 | -10 | -66 | -71 | -72 | -71 | -48 | -67 | -68 | -68 |
|  | 7 | 1 | -57 | -71 | -73 | -71 | -43 | n.a | n.a | n.a |
|  | 14 | 18 | -26 | -70 | -74 | -71 | -5 | -63 | -66 | -68 |
|  | 28 | 51 | -1 | -68 | -73 | -71 | 42 | -67 | -70 | -68 |
| 2: + CH4 | 28 | -20 | -72 | -71 | -73 | -72 | -71 | -70 | -72 | -68 |
| 3: + 13C-leu | 28 | 68 | -22 | -66 | -73 | -71 | 47 | -66 | -70 | -68 |
|  |  |  |  |  |  |  |  |  |  |  |
| AOM50 | 1: + CH4 + 13C-leu | 0 | -26 | -74 | -71 | -70 | -70 | n.a | n.a | n.a | n.a |
|  | 28 | 113 | -7 | -55 | -70 | -68 | n.a | n.a | n.a | n.a |
| 2: + CH4 | 28 | -34 | -74 | -71 | -71 | -70 | n.a | n.a | n.a | n.a |
| 3: + 13C-leu | 28 | 125 | -23 | -65 | -69 | -68 | n.a | n.a | n.a | n.a |

Table S3. Classification of metagenomic 16S rRNA gene fragments on phylogenetic levels of Class (normalized to 100%) in AOM37 and AOM60 (data from Krukenberg et al., 2018) as well as their dominant C assimilation pathway and signature lipids based on published literature or this study. The composition of the AOM60 culture is largely identical to the tested AOM50 culture. Both cultures contain the same ANME-1 and HotSeep-1 strains, and the cultures show similar activity at both temperatures.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **AOM37** | **AOM60** | **C assimilation** | **Signature lipids** |
| **Archaea** |  |  |  |  |
| ANME-1 | 56.3 | 39.8 | autotrophic | GDGT-0, -1, -2 (**A**) |
| Thermoplasmata | 0.9 | 4.9 | heterotrophic | Archaeol, GDGT-0 (**B**) |
| Lokiarchaeota | 0.4 |  | heterotrophic | Archaeol, GDGT-0, 1, 2 (**C**) |
| Bathyarchaeota | | 10.5 | heterotrophic | Archaeol, GDGT-0, -1, -2 (**D**) |
| Marine Hydrothermal Vent Group 1  (MHVG-1)\_unclassified | | 0.7 |  |  |
| Archaeoglobi | | 0.7 |  |  |
| Thermococci | | 0.4 |  |  |
| Thermoprotei | | 0.3 |  |  |
| Euryarchaeota\_unclassified | | 0.3 |  |  |
| Korarchaeota Incertae Sedis | | 0.3 |  |  |
| Terrestrial Hot Spring Group (THSCG) | | 0.3 |  |  |
| **Bacteria** |  |  |  |  |
| Deltaproteobacteria\_SEEP-SRB2 | 25.6 |  | autotrophic | C16:0, C18:1ω7, C18:0 (**E**) |
| Deltaproteobacteria\_Ca. D. auxilii | | 13.7 | autotrophic | C16:0, C18:0 (**E**) |
| Deltaproteobacteria\_others | 2.6 | 10.4 |  |  |
| Deferribacteres | 1.2 |  |  | |
| Spirochaetes | 5.0 |  | heterotrophic | *i*C15, *i*C17, C18:1ω7 (**F**) |
| Chloroflexi\_unclassified | 0.2 | 0.8 | heterotrophic |  |
| Anaerolineae | 2.2 | 3.7 | heterotrophic | *i*C15, *i*C17, C16:0 (**G**) |
| Phycisphaerae | | 1.5 | heterotrophic | C16:0, *i*C16, *i*C16:1 (**H**) |
| Candidate division JS1\_unclassified | 0.7 |  |  |  |
| Candidate division WS3\_unclassified | 0.6 |  |  |  |
| Candidate division KB1\_unclassified | 0.2 | 1.8 |  |  |
| Candidate division OP3\_unclassified | 0.3 |  |  |  |
| Candidate division OP8\_unclassified | | 0.3 |  |  |
| Thermotogae | 0.2 | 0.6 |  |  |
| AK8 |  | 0.4 |  |  |
| Proteobacteria\_unclassified | | 1.6 |  |  |
| Synergistia | 0.2 |  |  |  |
| **Unclassified** | 2 | 6.5 |  |  |

**A**: (Schouten et al., 2001; Blumenberg et al., 2004; Elvert et al., 2005); **B**: (Yoshinaga et al., 2015; Yin et al., 2022); **C**: (Imachi et al., 2020); **D**: (Yu et al., 2018); **E**: (this study); **F**: (Livermore and Johnson, 1974; Vishnuvardhan Reddy et al., 2013); **G**: (Yamada et al., 2006); **H**: (Fukunaga et al., 2009)

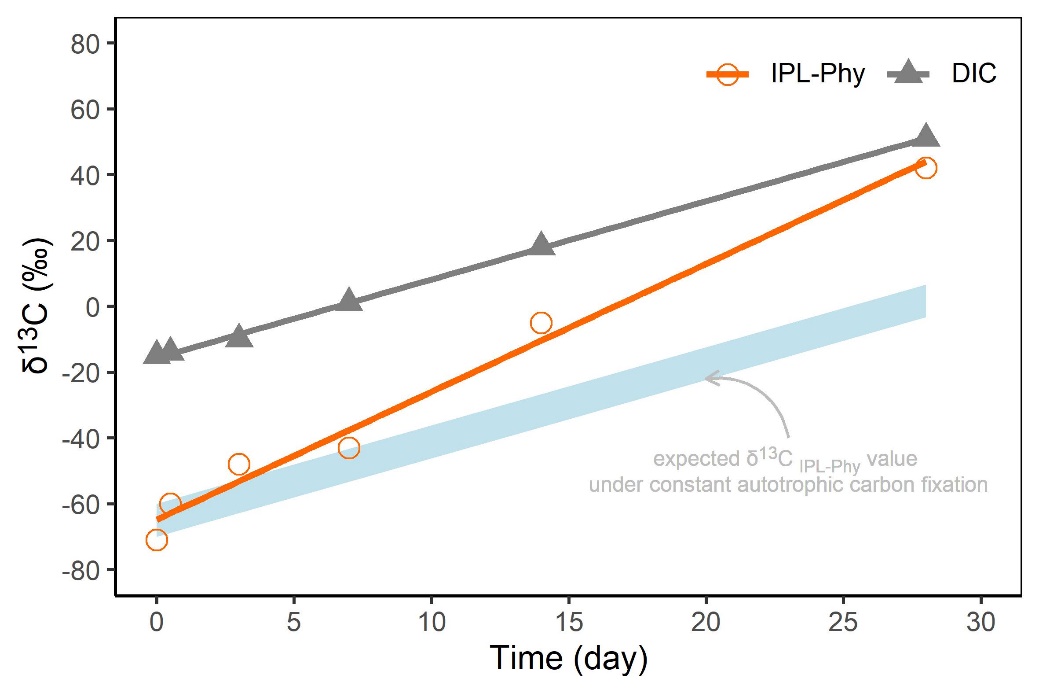


Fig. S1. Development of δ13C values (in ‰) of DIC and IPL-Phy during incubation of AOM37 over 28 days in experiment 1. The stronger increasing δ13C values of IPL-Phy opposed to DIC indicate a contribution of carbon used for archaeal lipid synthesis being different to DIC. The expected offset is displayed by the light blue bar assuming constant isotopic fractionation during autotrophic carbon fixation of the ANMEs.

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