

1 Supporting Information for Geochemical, biological and clumped isotopologue  
2 evidence for substantial microbial methane production under carbon  
3 limitation in serpentinites of the Samail ophiolite, Oman

4 Daniel B. Nothaft<sup>a,\*</sup>, Alexis S. Templeton<sup>a,\*</sup>, Jeemin H. Rhim<sup>b</sup>, David T. Wang<sup>b,1</sup>, Jabrane Labidi<sup>c</sup>,  
5 Hannah M. Miller<sup>a,2</sup>, Eric S. Boyd<sup>d</sup>, Juerg M. Matter<sup>e</sup>, Shuhei Ono<sup>b</sup>, Edward D. Young<sup>c</sup>, Sebastian H.  
6 Kopf<sup>a</sup>, Peter B. Kelemen<sup>f</sup>, Mark E. Conrad<sup>g</sup>, The Oman Drilling Project Science Team

7 <sup>a</sup>*Department of Geological Sciences, University of Colorado, Boulder, CO, USA*

8 <sup>b</sup>*Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge,  
9 Massachusetts, USA*

10 <sup>c</sup>*Department of Earth, Planetary, and Space Sciences, University of California, Los Angeles, CA, USA*

11 <sup>d</sup>*Department of Microbiology & Immunology, Montana State University, Bozeman, MT*

12 <sup>e</sup>*National Oceanography Centre, University of Southampton, Southampton, UK*

13 <sup>f</sup>*Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY, USA*

14 <sup>g</sup>*Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

---

---

15 **Contents**

16 **S1 Text S1. Overview of CH<sub>4</sub> dynamics in other states of the system: wells WAB56,**  
17 **WAB71, and CM2A** **2**

18 **S2 Figures** **5**

19 **S3 References** **8**

20 **List of Figures**

21 **S1 Topographic Map, Wadi Tayin, Oman.** . . . . . **5**

22 **S2 Oman well water stable isotopic composition** . . . . . **6**

23 **S3 16S rRNA gene read heat map, Oman 2018.** . . . . . **7**

24 **S4  $\epsilon_{\text{methane/water}}$  and  $\Delta^{13}\text{CH}_3\text{D}$  plot.** . . . . . **8**

---

\*Corresponding authors

*Email addresses:* [daniel.nothaft@colorado.edu](mailto:daniel.nothaft@colorado.edu) (Daniel B. Nothaft), [alexis.templeton@colorado.edu](mailto:alexis.templeton@colorado.edu) (Alexis S. Templeton)

<sup>1</sup>Current address: ExxonMobil Upstream Research Company, Spring, TX 77389, USA

<sup>2</sup>Current address: Itasca Denver, Inc., 143 Union Blvd. Suite 525 Lakewood, CO 80228, USA

25 **S1. Text S1. Overview of CH<sub>4</sub> dynamics in other states of the system: wells WAB56, WAB71,**  
26 **and CM2A**

27 Here, we briefly discuss three more wells to provide a broader perspective on CH<sub>4</sub> dynamics in the Samail  
28 ophiolite. The first of these wells is WAB56, which is situated in a catchment dominated by harzburgite  
29 (Main Text Figure 1; Figure S1; Main Text Table 1; also, WAB56 is pictured in Figure 1b of Rempfert  
30 *et al.* (2017)). Thus, the hydrogeologic setting of WAB56 is similar to that of NSHQ14. Yet, in comparison  
31 to fluids from NSHQ14, fluids sampled from WAB56 in 2015, 2016, and 2017 had lower pH,  $c_{\sum Ca}$ ,  $c_{H_2}$ ,  
32  $c_{CH_4}$ , and  $\delta^{13}C_{CH_4}$  (Main Text Table 1, Main Text Table 3, Main Text Table 4; Rempfert *et al.*, 2017). For  
33 example, fluids sampled from WAB56 in 2015 had a pH of 10.6,  $c_{\sum Ca}$  of  $430 \mu\text{mol} \cdot \text{L}^{-1}$ , and  $c_{H_2}$  below  
34 quantifiable levels (Rempfert *et al.*, 2017). Differences in fluid chemistry between WAB56 and NSHQ14  
35 could be related to well construction. While NSHQ14 was drilled to 304 m depth and is cased only to 5.8 m,  
36 WAB56 was drilled to 106 m depth and is fully cased with a screened interval from 7 mbgl to 27 mbgl (Main  
37 Text Table 1). Thus, the fluids sampled from WAB56 may have derived from a shallow aquifer containing  
38 fluids of relatively short residence time, or a mixture of deep and shallow peridotite-reacted waters. Fluids  
39 sampled from WAB56 in 2015 had  $\delta^{13}C_{CH_4}$  of  $-83.2 \text{‰}$  VPDB and 4 % of 16S rRNA gene reads of subsurface  
40 biomass affiliated with *Methanobacterium*, which is remarkably similar to fluids sampled from WAB188 in  
41 the same year ( $\delta^{13}C_{CH_4}$  of  $-71.3 \text{‰}$  VPDB and 8 % of 16S rRNA gene reads of subsurface biomass assigned to  
42 *Methanobacterium*) (Main Text Table 4; Rempfert *et al.*, 2017). Thus, it seems that the casing construction  
43 at WAB56 has restricted sampling to shallow waters of relatively low pH,  $c_{Ca^{2+}}$ , and  $c_{H_2}$ , where conditions  
44 of H<sub>2</sub> limitation and excess  $\sum CO_2$  allow the classic isotope effect of hydrogenotrophic methanogenesis to  
45 be expressed, as at WAB188.

46 Well WAB71 is located 2.4 km north of NSHQ04, and is set in dunite, 30 m east of a faulted contact  
47 with harzburgite (Main Text Figure 1; Figure S1; Main Text Table 1). It is similar to NSHQ04 in aqueous  
48 chemical composition ( $Ca^{2+} - OH^-$  water; Main Text Table 2), dissolved gas composition (high CH<sub>4</sub>/H<sub>2</sub> and  
49 C<sub>1</sub>/(C<sub>2</sub> + C<sub>3</sub>) ratios; Main Text Figure 2, Main Text Table 3),  $\delta^{13}C_{CH_4}$  (interannual mean of  $+3.3 \text{‰}$  VPDB,  
50  $s = 0.5 \text{‰}$ ,  $n = 4$ ; Main Text Figure 3a; Main Text Table 4), and CH<sub>4</sub>-cycling microbial community  
51 composition. The dominant CH<sub>4</sub>-cycling taxon at WAB71 appears to be *Methylococcus*, which accounted  
52 for 1 % of 16S rRNA gene reads of DNA extracted from subsurface biomass obtained in 2018 from WAB71  
53 (Figure S3), which is consistent with prior sampling at this well (mean 2015 through 2018 of 1 %; Rempfert  
54 *et al.*, 2017; Kraus *et al.*, 2018). 16S rRNA gene reads affiliated with *Methanobacterium* were not detected  
55 at WAB71 in this study of samples obtained in 2018, but were found in low relative abundance ( $< 1 \text{‰}$   
56 of reads) in samples from 2015 and 2017 (Rempfert *et al.*, 2017; Kraus *et al.*, 2018). The chemical and  
57 microbial similarities between WAB71 and NSHQ04 suggest that CH<sub>4</sub> cycle processes at WAB71 may be  
58 similar to those at NSHQ04, where a dominantly microbial source of CH<sub>4</sub> and an important role of aerobic

59 CH<sub>4</sub> oxidation were inferred. However, this conclusion is more speculative at WAB71, since it was not  
 60 feasible to measure clumped isotopologue relative abundances in CH<sub>4</sub> from WAB71 by the methods of this  
 61 study due to the relatively lower  $c_{\text{CH}_4}$  at WAB71 ( $7.76 \mu\text{mol} \cdot \text{L}^{-1}$  in 2018,  $14.8 \mu\text{mol} \cdot \text{L}^{-1}$  in 2017; Main  
 62 Text Table 3). In addition, some aspects of WAB71 differ from NSHQ04. These include that WAB71 fluids  
 63 have higher pH by 0.7 and lower  $\delta\text{D}_{\text{CH}_4}$  (interannual mean of  $-310 \text{‰}$  VSMOW,  $s = 4 \text{‰}$ ,  $n = 2$ ; Main Text  
 64 Figure 3a; Main Text Table 4). In addition, WAB71 had the lowest  $Eh$  ( $-229 \text{ mV}$ ; main text Table 1) and  
 65  $c_{\text{SO}_4^{2-}}$  ( $60.8 \mu\text{mol} \cdot \text{L}^{-1}$ ) measured in groundwaters sampled in 2018. The reduced character and low  $c_{\text{SO}_4^{2-}}$   
 66 of groundwaters sampled from WAB71 may be indicative of microbial  $\text{SO}_4^{2-}$  reduction at this well. Indeed,  
 67 DNA extracted from subsurface samples from WAB71 had the highest 16S rRNA gene relative abundance  
 68 of class Thermodesulfobionia among wells sampled in 2018 (20%; Figure S3; also mean of 19% from 2015  
 69 to 2018; Rempfert *et al.*, 2017; Kraus *et al.*, 2018). Cultured representatives of Thermodesulfobionia are  
 70 capable of  $\text{SO}_4^{2-}$  reduction coupled to H<sub>2</sub> oxidation and may additionally/alternatively oxidize C<sub>1</sub>-C<sub>3</sub> acids  
 71 and use thiosulfate, sulfite, Fe<sup>3+</sup> or NO<sub>3</sub><sup>-</sup> as terminal electron acceptors for anaerobic respiration (Henry  
 72 *et al.*, 1994; Sekiguchi *et al.*, 2008). Further, 16S rRNA gene sequences affiliated with ANME-1b have been  
 73 detected in DNA from WAB71, albeit in low relative gene abundance ( $< 1 \%$  of reads in 2018; Main Text  
 74 Figure 5; the same is true of samples from 2017 and 2015, but ANME-1b was not detected in 2016; Rempfert  
 75 *et al.*, 2017; Kraus *et al.*, 2018). These data suggest that  $\text{SO}_4^{2-}$ -reducing bacteria may have contributed to  
 76 the low  $c_{\text{H}_2}$  at WAB71, which may have allowed  $\text{SO}_4^{2-}$ -driven CH<sub>4</sub> oxidation by ANME-1b to be energetically  
 77 competitive at this well, whereas it is apparently not in other sampled wells. The lower  $\delta\text{D}_{\text{CH}_4}$  at WAB71  
 78 relative to NSHQ04 could be due to a combination of aerobic and anaerobic methanotrophy at WAB71,  
 79 which may have different C and H isotope effects than aerobic methanotrophy alone.

80 Well CM2A was drilled in the crust-mantle transition zone of the ophiolite by the Oman Drilling Project  
 81 in late 2017 (Main Text Figure 1; Figure S1). Drill cuttings from the rotary-drilled well, CM2A, and cores  
 82 from the adjacent diamond-wireline drilled well, CM2B, contain mostly dunite, with occasional gabbro and  
 83 harzburgite (Main Text Table 1). Of the 300 m of core retrieved from CM2B, chromitite was noted only in  
 84 one 30 cm-thick layer at 115 m depth. The water level in CM2A was 13.4 m, so these deep chromitites should  
 85 be water-saturated and therefore not catalytic for CO<sub>2</sub> reduction to CH<sub>4</sub>. CM2A contains Ca<sup>2+</sup> – OH<sup>-</sup>  
 86 waters with pH and major ion chemistry similar to NSHQ14 (Main Text Table 1; Main Text Table 2). Fluids  
 87 sampled from CM2A had a  $c_{\text{CH}_4}$  of  $152 \mu\text{mol} \cdot \text{L}^{-1}$ , which is almost double that of NSHQ14, concentrations of  
 88 C<sub>2</sub> – C<sub>4</sub> *n*-alkanes up to  $4.11 \cdot 10^{-2} \mu\text{mol} \cdot \text{L}^{-1}$ , which is similar to or somewhat lower than those of NSHQ14,  
 89 and a  $c_{\text{H}_2}$  of  $3.38 \mu\text{mol} \cdot \text{L}^{-1}$ , which is 50 times lower than NSHQ14, although still higher than all other  
 90 wells sampled in 2018 (Main Text Figure 2; Main Text Table 3). In comparison to NSHQ14 and NSHQ04,  
 91 CH<sub>4</sub> in fluids sampled from CM2A had lower  $\delta^{13}\text{C}$  (inter-laboratory mean of  $-4.3 \text{‰}$  VPDB; Main Text  
 92 Table 4; Main Text Figure 3a). CH<sub>4</sub> from CM2A had the highest  $\delta\text{D}$  of all studied wells (inter-laboratory  
 93 mean of  $-198 \text{‰}$  VSMOW; Main Text Table 4; Main Text Figure 3a). Values of  $\varepsilon_{\text{methane/water}}$ ,  $\Delta^{13}\text{CH}_3\text{D}$ ,

94 and  $\Delta^{12}\text{CH}_2\text{D}_2$  indicate that  $\text{CH}_4$  from CM2A is not in isotopic equilibrium with water, nor intramolecular  
95 equilibrium (Main Text Figure 3b and d; Main Text Table 4). 16S rRNA gene sequences affiliated with  
96 *Methanobacterium* were detected in DNA extracted from biomass in waters pumped from CM2A at low  
97 relative abundances ( $< 1\%$  of reads; Figure S3). No sequences affiliated with methanotrophs were detected  
98 in DNA extracted from CM2A fluids (Main Text Figure 5). CM2A and NSHQ14 fluids share several  
99 relevant characteristics including isotopic disequilibrium in  $\text{CH}_4$ , detection of *Methanobacterium*, similar  
100 concentrations of major ionic species, and apparent scarcity of potentially catalytic, water-unsaturated  
101 chromitites. These similarities suggest that  $\text{CH}_4$  in CM2A, like NSHQ14, is dominantly a mixture of abiotic  
102  $\text{CH}_4$  being released from fluid inclusions and microbial  $\text{CH}_4$ .

103 S2. Figures

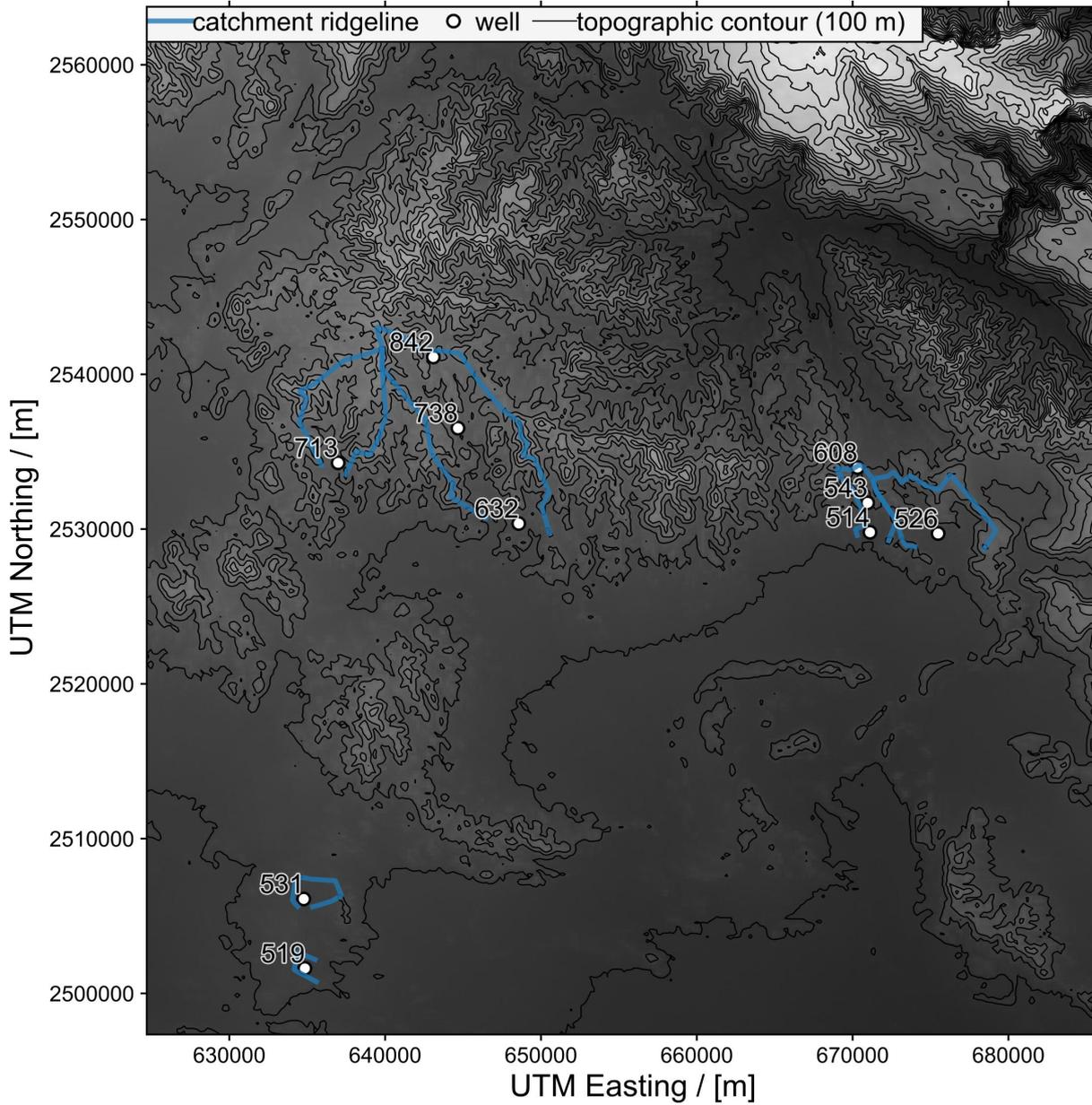


Figure S1: Study area in Samail ophiolite, Sultanate of Oman. Digital elevation model from [USGS \(2010\)](#). Elevations in meters above sea level are listed on the upper left of well location markers. Colors are scaled by elevation from low (dark) to high (light). Ridgelines of hydrologic catchments were estimated from the topography. If multiple wells shared catchment area, one catchment is depicted for visual clarity (i.e. WAB71, NSHQ04, and WAB188 share catchment area that is separate from NSHQ14; see Main Text Figure 1 for well names).

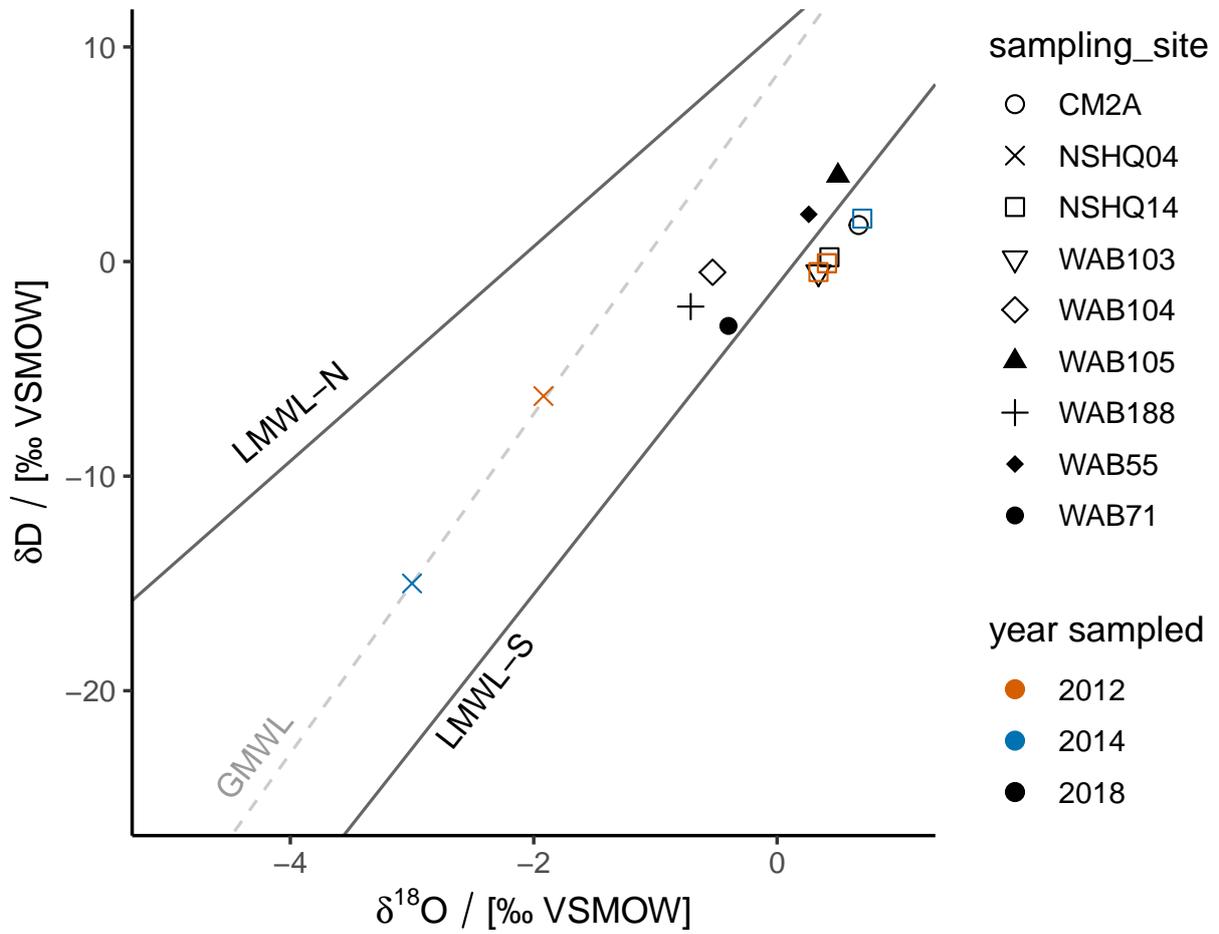


Figure S2: Plot of Oman groundwater stable isotopic compositions. Samples from 2012 were reported in [Vankeuren et al. \(2019\)](#). Samples from 2014 reported in [Miller et al. \(2016\)](#). Abbreviations: LMWL-N and LMWL-S, Oman local meteoric water lines derived from northern and southern sources, respectively ([Weyhenmeyer et al., 2002](#)); GMWL, global meteoric water line ([Terzer et al., 2013](#)).

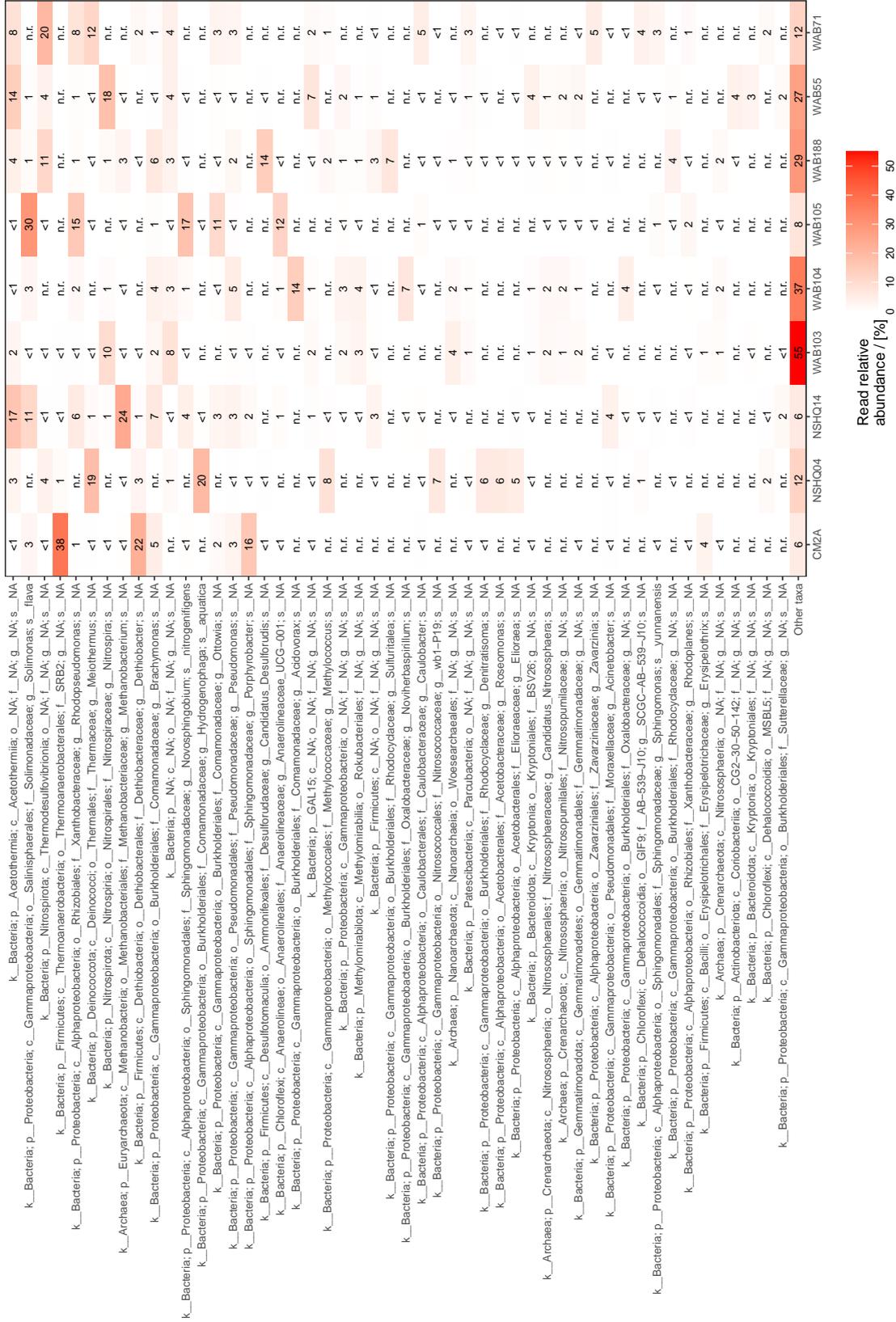


Figure S3: Heat map of the top 50 16S rRNA gene taxonomic assignments in samples obtained in 2018, arranged in descending order of abundance. Read relative abundances are reported as percentages rounded to the ones place. Cases when a taxon was detected in a sample and was < 1% read relative abundance after rounding are labeled “< 1”. Cases when no reads of a taxon were detected in a sample are labeled “n.r.”

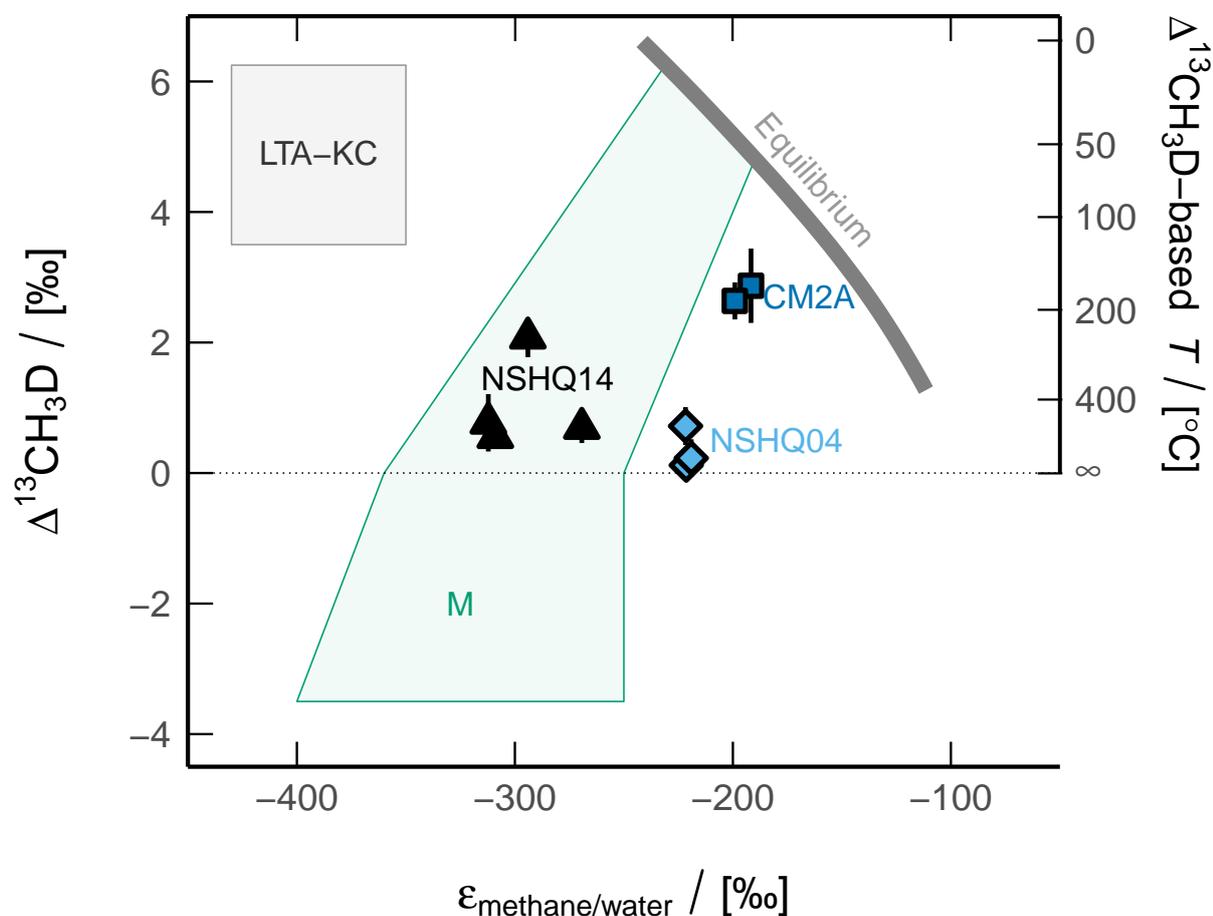


Figure S4:  $\epsilon_{\text{methane/water}}$  and  $\Delta^{13}\text{CH}_3\text{D}$  plot of methane from Oman well waters, after Wang et al. (2015). Equilibrium (thick, light gray line) from Horibe and Craig (1995) and Young et al. (2017). Abbreviations: LTA-KC, low-temperature abiotic (Kidd Creek-type); M, microbial.

### 104 S3. References

- 105 Henry, E.A., Devereux, R., Maki, J.S., Gilmour, C.C., Woese, C.R., Mandelco, L., Schauder, R., Remsen, C.C.,  
 106 Mitchell, R.. Characterization of a new thermophilic sulfate-reducing bacterium. Arch Microbiol 1994;161(1):62–69. URL:  
 107 <https://doi.org/10.1007/BF00248894>. doi:10.1007/BF00248894.
- 108 Horibe, Y., Craig, H.. DH fractionation in the system methane-hydrogen-water. Geochim Cosmochim Acta 1995;59(24):5209–  
 109 5217. doi:10.1016/0016-7037(95)00391-6.
- 110 Kraus, E.A., Stamps, B.W., Rempfert, K.R., Nothaft, D.B., Boyd, E.S., Matter, J.M., Templeton, A.S., Spear, J.R..  
 111 Biological methane cycling in serpentinization-impacted fluids of the Samail ophiolite of Oman. AGU Fall Meeting Abstracts  
 112 2018;.
- 113 Miller, H.M., Matter, J.M., Kelemen, P., Ellison, E.T., Conrad, M.E., Fierer, N., Ruchala, T., Tominaga, M.,  
 114 Templeton, A.S.. Modern water/rock reactions in Oman hyperalkaline peridotite aquifers and implications for microbial  
 115 habitability. Geochim Cosmochim Acta 2016;179:217 – 241. URL: [http://www.sciencedirect.com/science/article/pii/](http://www.sciencedirect.com/science/article/pii/S0016703716300205)  
 116 [S0016703716300205](http://www.sciencedirect.com/science/article/pii/S0016703716300205). doi:10.1016/j.gca.2016.01.033.

- 117 Rempfert, K.R., Miller, H.M., Bompard, N., Nothaft, D., Matter, J.M., Kelemen, P., Fierer, N., Templeton, A.S.  
118 Geological and geochemical controls on subsurface microbial life in the Samail Ophiolite, Oman. *Front Microb* 2017;8(56):1–  
119 21. doi:10.3389/fmicb.2017.00056.
- 120 Sekiguchi, Y., Muramatsu, M., Imachi, H., Narihiro, T., Ohashi, A., Harada, H., Hanada, S., Kamagata, Y.. Ther-  
121 modesulfovibrio aggregans sp. nov. and Thermodesulfovibrio thiophilus sp. nov., anaerobic, thermophilic, sulfate-reducing  
122 bacteria isolated from thermophilic methanogenic sludge, and emended description of the genus Thermodesulfovibrio. *Int*  
123 *J Syst Evol Microbiol* 2008;58(11):2541–2548. URL: [https://www.microbiologyresearch.org/content/journal/ijsem/10.](https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijvs.0.2008/000893-0)  
124 [1099/ijvs.0.2008/000893-0](https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijvs.0.2008/000893-0). doi:10.1099/ijvs.0.2008/000893-0.
- 125 Terzer, S., Wassenaar, L.I., Araguás-Araguás, L.J., Aggarwal, P.K.. Global isoscapes for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in precipitation:  
126 improved prediction using regionalized climatic regression models. *Hydrol Earth Syst Sci* 2013;17(11):4713–4728. URL:  
127 <https://www.hydrol-earth-syst-sci.net/17/4713/2013/>. doi:10.5194/hess-17-4713-2013.
- 128 USGS, . Digital Elevation - Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010). 2010. doi:/10.5066/F7J38R2N.
- 129 Vankeuren, A.N.P., Matter, J.M., Stute, M., Kelemen, P.B.. Multitracer determination of apparent groundwater ages in  
130 peridotite aquifers within the Samail ophiolite, Sultanate of Oman. *Earth Planet Sci Lett* 2019;516:37–48. doi:10.1016/j.  
131 [eps1.2019.03.007](https://doi.org/10.1016/j.epsl.2019.03.007).
- 132 Wang, D.T., Gruen, D.S., Lollar, B.S., Hinrichs, K.U., Stewart, L.C., Holden, J.F., Hristov, A.N., Pohlman, J.W., Morrill,  
133 P.L., Könneke, M., et al. Nonequilibrium clumped isotope signals in microbial methane. *Science* 2015;348(6233):428–431.  
134 doi:10.1126/science.aaa4326.
- 135 Weyhenmeyer, C.E., Burns, S.J., Waber, H.N., Macumber, P.G., Matter, A.. Isotope study of moisture sources, recharge  
136 areas, and groundwater flow paths within the eastern Batinah coastal plain, Sultanate of Oman. *Water Resources Research*  
137 2002;38(10). doi:10.1029/2000WR000149.
- 138 Young, E., Kohl, I., Lollar, B.S., Etiope, G., Rumble Iii, D., Li, S., Haghnegahdar, M., Schauble, E., McCain, K.,  
139 Foustoukos, D., et al. The relative abundances of resolved  $^{12}\text{CH}_2\text{D}_2$  and  $^{13}\text{CH}_3\text{D}$  and mechanisms controlling isotopic bond  
140 ordering in abiotic and biotic methane gases. *Geochim Cosmochim Acta* 2017;203:235–264. doi:10.1016/j.gca.2016.12.041.