Aqueous geochemical and microbial variation across discrete depth intervals in a peridotite aquifer assessed using a packer system in the Samail Ophiolite, Oman

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Abstract

The potential for molecular hydrogen (H-OH⁻ groundwaters bearing up to 4.05 μ mol[?]L⁻¹ H₂, 3.81 μ mol[?]L⁻¹ methane (CH₄) and 946 μ mol[?]L⁻¹ sulfate (SO₄²⁻) revealed an ecosystem dominated by Bacteria affiliated with the class Thermodesulfovibrionia, a group of chemolithoheterotrophs supported by H₂ oxidation coupled to SO₄²⁻ reduction. In shallower, oxidized Mg²⁺-HCO₃⁻ groundwaters, aerobic and denirifying heterotrophs were relatively more abundant. High δ^{13} C and δ D of CH₄ (up to 23.9 CH₄ oxidation, particularly in Ca²⁺-OH⁻ waters with evidence of mixing with Mg²⁺-HCO₃⁻ waters. This study demonstrates the power of spatially resolving groundwaters to probe their distinct geochemical conditions and chemosynthetic communities. Such information will help improve predictions of where microbial activity in fractured rock ecosystems might occur, including beyond Earth.

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13 Key Points:

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•	Packers	were used	to sample	groundwaters	from d	iscrete 1	peridotite	aquifers.
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- The discrete aquifers contained waters with distinct chemical compositions and microbial communities.
- Chemolithoheterotrophic sulfate reduction was a dominant metabolic strategy in ferred from 16S rRNA gene homology.

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19 Abstract

The potential for molecular hydrogen (H_2) generated via serpentinization to fuel sub-20 surface microbial ecosystems independent from photosynthesis has prompted biogeochem-21 ical investigations of serpentinization-influenced fluids. However, investigations typically 22 sample via surface seeps or open-borehole pumping, which can mix chemically distinct 23 waters from different depths. Depth-indiscriminate sampling methods could thus hin-24 der understanding of the spatial controls on nutrient availability for microbial life. To 25 resolve distinct groundwaters in a low-temperature serpentinizing environment, we de-26 ployed packers (tools that seal against borehole walls during pumping) in two 400 m-deep. 27 peridotite-hosted wells in the Samail Ophiolite, Oman. Isolation and pumping of discrete 28 intervals as deep as 108 m to 132 m below ground level revealed multiple aguifers that 29 ranged in pH from 8 to 11. Chemical analyses and 16S rRNA gene sequencing of deep, 30 highly-reacted $Ca^{2+}-OH^{-}$ groundwaters bearing up to $4.05 \,\mu \text{mol} \cdot \text{L}^{-1} \text{ H}_2$, $3.81 \,\mu \text{mol} \cdot \text{L}^{-1}$ methane (CH₄) and 946 $\mu \text{mol} \cdot \text{L}^{-1}$ sulfate (SO₄²⁻) revealed an ecosystem dominated 31 32 by Bacteria affiliated with the class Thermodesulfovibrionia, a group of chemolithoheterotrophs supported by H_2 oxidation coupled to SO_4^{2-} reduction. In shallower, oxidized Mg^{2+} -33 34 HCO_3^- groundwaters, aerobic and denitrifying heterotrophs were relatively more abun-35 dant. High δ^{13} C and δ D of CH₄ (up to 23.9% VPDB and 45% VSMOW, respectively), indicated microbial CH₄ oxidation, particularly in Ca²⁺ – OH⁻ waters with evidence of mixing with Mg²⁺–HCO₃⁻ waters. This study demonstrates the power of spatially 36 37 38 resolving groundwaters to probe their distinct geochemical conditions and chemosynthetic 39 communities. Such information will help improve predictions of where microbial activ-40 ity in fractured rock ecosystems might occur, including beyond Earth. 41

⁴² Plain Language Summary

Peridotite rocks can react with water to form hydrogen gas. Microbes can combine 43 hydrogen with oxidants to power their cells. Rocks similar to peridotite have been abun-44 dant throughout the history of Earth and the Solar System. Therefore, peridotite-water 45 interaction is important for understanding the history and distribution of life. Prior stud-46 ies investigating these processes have sampled waters from the surface of peridotite ex-47 posures or from open wells. These sampling methods risk contaminating deep, peridotite-48 hosted waters with shallower waters influenced by the atmosphere. In this study, we used 49 packers (tools that can be used to pump waters from separate regions of the subsurface 50 in isolation) to better understand the distribution of microbes and nutrients in subsur-51 face peridotites. We sampled waters from separate subsurface zones as deep as 108 m to 52 132 m in two wells in peridotite. Waters from different depths had distinct chemical com-53 positions and microbial communities. Sulfate reducing bacteria were dominant in wa-54 ters that had most extensively reacted with peridotite in isolation, while microbes that 55 consume nitrate or oxygen were also prevalent in waters with more evidence of atmo-56 spheric influence. The advanced sampling techniques we used help to distinguish where 57 and how microbes live in the subsurface. 58

59 1 Introduction

Serpentinization reactions between peridotite and water can generate molecular hy-60 drogen (H₂), and drive the reduction of carbon dioxide (CO₂) to organic acids such as 61 formate (HCOO⁻), and methane (CH₄) (Neal & Stanger, 1983; McCollom & Bach, 2009; 62 McCollom & Seewald, 2003; Miller et al., 2017; Etiope et al., 2018; Klein et al., 2019), 63 powerful electron donors that may fuel non-photosynthetic, subsurface microbial com-64 munities (Nealson et al., 2005). The potential relevance of such ecosystems to subsur-65 face life on the modern and early Earth, as well as life beyond Earth, has led to numer-66 ous biogeochemical investigations of groundwaters from peridotite aquifers. These stud-67 ies have primarily focused on groundwater samples from surface seeps or open-well pump-68

ing (W. Brazelton et al., 2012; Chavagnac, Monnin, et al., 2013; Chavagnac, Ceuleneer, 69 et al., 2013; Morrill et al., 2013; Suzuki et al., 2013, 2014; Crespo-Medina et al., 2014; 70 Meyer-Dombard et al., 2015; Woycheese et al., 2015; Postec et al., 2015; Rempfert et al., 71 2017; Canovas III et al., 2017; W. J. Brazelton et al., 2017; Crespo-Medina et al., 2017; 72 Suzuki et al., 2017; Marques et al., 2018; Nothaft et al., 2020; Leong et al., 2020). While 73 these studies have transformed the understanding of the chemistry and biology of peridotite-74 hosted ecosystems, the sampling methods tend to yield mixtures of deep fluids with at-75 mospherically influenced shallow fluids that were hydrologically segregated prior to sam-76 pling. This, in turn, can make it difficult to differentiate the sources, residence times, 77 and geochemical states of fluids in serpentinizing systems, all of which influence the avail-78 ability of nutrients to support endogenous microbial communities. For example, sam-79 ples may be mixtures of surface-influenced groundwaters containing dissolved inorganic 80 carbon $(\sum CO_2)$, O_2 , and nitrate (NO_3^-) and deeper groundwaters rich in H_2 , CH_4 , and 81 sulfate (SO_4^{2-}) . Downstream analyses of samples of potentially mixed origin therefore 82 may obscure understanding of the subsurface peridotite-hosted biosphere. 83

The few hydrologic studies of ophiolite aquifers conducted to date show that ground-84 water flow is fracture-dominated, that fractures occur on multiple spatial scales, and that 85 they are heterogeneously distributed, although generally more abundant within $\sim 50\,\mathrm{m}$ 86 to $\sim 100 \,\mathrm{m}$ of the surface (Boronina et al., 2003; Dewandel et al., 2005; Segadelli et al., 87 2017; Jeanpert et al., 2019; Lods et al., 2020). When deep, long-residence time ground-88 waters approach the surface, they have the potential to mix with lower-residence time 89 groundwaters hosted in shallow, relatively high-transmissivity fracture networks (Paukert 90 Vankeuren et al., 2019). This mixing may often be overlooked because groundwater pH 91 is commonly used as an indicator of the extent of reaction of the water with peridotite out of contact with the atmosphere (with highly reacted waters being hyperalkaline, of-93 ten defined as pH > 11), but pH is weakly sensitive to mixing of hyperalkaline ground-94 waters with circumneutral to moderately alkaline (pH 7 to 9) groundwaters derived from 95 water-rock reaction in contact with the atmosphere (Leong et al., 2020). 96

In this study, we directly assessed the relationship between spatially heterogeneous 97 hydrogeochemical parameters and subsurface microbial community compositions in ophi-98 olite aquifers through the use of packers (tools that can be inflated at determined depths 99 to seal against borehole walls during pumping). The use of packers allowed us to sam-100 ple groundwaters from discrete, isolated depth intervals within two 400 m-deep wells in 101 the Samail Ophiolite, Oman. We obtained depth-resolved aqueous geochemical data from 102 both downhole wireline logging and measurements of solute concentrations in ground-103 water samples pumped from defined packer intervals. Gases (H₂, CO, and alkanes) dis-104 solved in the pumped groundwaters were analyzed for their aqueous concentrations and 105 their stable isotopic compositions (CH_4 and C_2H_6 only). Microbial community compo-106 sitions of filter-concentrated biomass were assessed through 16S rRNA gene sequencing 107 of extracted DNA. We accessed discrete aquifers at multiple depths, which ranged in pH 108 from 8 to 11 and hosted distinct microbial communities. The deployment of packers to 109 probe the biogeochemistry of subsurface, peridotite-hosted aquifers as presented herein 110 marks a considerable advance in the ability to sample deep, serpentinization-influenced 111 fluids isolated from surficial fluids and assess the effect of mixing these fluids on micro-112 bial processes. In doing so, this study furthers understanding of where and how serpentinization-113 influenced ecosystems may occur in the subsurface of Earth and celestial rocky bodies. 114

¹¹⁵ 2 Site and methods

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2.1 Site description and drilling

The Oman Drilling Project established a multi-borehole observatory (MBO) in Wadi Lawayni in the Wadi Tayin massif of the Samail Ophiolite (P. Kelemen et al., 2013, 2020). The initial hydrological characterization of the MBO has been previously described in

detail (Lods et al., 2020). Here, we focus on two 400 m deep, 6-inch diameter, rotary-120 drilled wells, BA1A and BA1D (Figure S1). Well BA1A was drilled in 2017, from Febru-121 ary 20th to March 2nd, and BA1D was drilled in 2018, from February 24th to March 15th. 122 These wells are situated in the mantle section of the ophiolite, 3 km north of the crust-123 mantle transition zone, and are spaced 15 m apart from one another. Shallow alluvium 124 was isolated from the boreholes by installing casing during drilling. The casing extends 125 to 21 m below ground level in BA1A and 26 m below ground level in BA1D (Figure 1). 126 Below the surficial alluvium, drill cuttings from these wells were predominantly fully ser-127 pentinized dunite in the upper 160 m to 250 m and partially serpentinized harzburgite 128 at greater depths (Figure 1; (P. Kelemen et al., 2020)). The drilling fluid was a mixture 129 of 1 volume percent "DrillFoam," a biodegradable sodium alcohol ethoxyl sulfate with 130 chelating agents, and 99 volume percent fresh water sourced from wells elsewhere in the 131 region. BA1A and BA1D were air lift tested immediately after well completion for well 132 development. The air lift tests involved pumping a mixture of water and air into the well 133 at different depths and monitoring the air-lifted discharge. 134



Figure 1. Biogeochemical trends with depth in BA1A and BA1D. *Eh*, pH, and electrical conductivity from well logs and pumped samples. All depths referenced to ground level. Well log data above casing base not shown. Well lithology from P. Kelemen et al. (2020). Hydraulically conductive zones sampled in this study had hydraulic conductivities of 10^{-6} m s^{-1} to 10^{-2} m s^{-1} (Lods et al., 2020).

2.2 Fluid sampling and field measurements

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¹³⁶ Downhole wireline logs (Matter et al., 2018) were obtained using an ALT QL40 OCEAN ¹³⁷ multi parameter probe, from which temperature, electrical conductivity, pH, and oxidation-¹³⁸ reduction potential are reported with accuracy/precision of $0.005/0.001 \,^{\circ}\text{C}$, $5/0.1 \,\mu\text{S} \cdot$ ¹³⁹ cm⁻¹, 0.01/0.001, and $1/0.1 \,\text{mV}$, respectively. BA1A was logged on April 22nd, 2017 and ¹⁴⁰ March 16th, 2018. BA1D was logged on March 19th, 2018 and February 13th, 2019.

The packer system (Solexperts) includes two inflatable rubber bladders ("packers") and a submersible pump (Grundfos SQE 1–140) (Lods et al., 2020). Inflating one or both of the packers at depth in a well enables the isolation of discrete subsurface intervals for targeted pumping. The depth intervals from which samples were collected in this study are reported in Table 1. The sampling setup is pictured in Figure S2. At least the volume of water in subsurface pipes was pumped and discarded prior to taking samples of groundwater. Temperature, pH, electrical conductivity, and oxidation-reduction potential (Eh), were monitored with probes at the pump outflow before and during sampling. The values of these parameters reported in Table 1 reflect the last measurements before the start of sampling for geochemical and microbiological analyses. Shorthand sample identifiers used throughout this manuscript consist of a well name, sampling year, and sampling interval, all concatenated (see Tables 1 and 2).

Well	Sa Year	mpling da Month	ite Day	Sampling Top	interval / [m] Bottom	Initial depth to water / [m]	$\begin{array}{c} {\rm Conductivity} \ / \\ \left[{\rm mS} \cdot {\rm cm}^{-1} \right] \end{array}$	Temperature / [°C]	$_{\rm pH}$	Eh/ [mV]
	2018	2	2 8	$55 \\ 100$	66 400 (open)	13.47	0.353 0.820	35.7 37.5	$8.71 \\ 10.69$	126 n.d.
BAIA	2019	1	14 16 16	0 (open) 41 108	$30 \\ 65 \\ 132$	17.25	$0.458 \\ 0.402 \\ 0.871$	$34.9 \\ 35.0 \\ 36.5$	8.10 8.21 10.67	$128 \\ 120. \\ -249$
BA1D	2019	1	11 13	$45 \\ 102$	75 132	17.03	1.40 1.72	34.6 35.2	$\begin{array}{c} 10.86\\ 11.01 \end{array}$	$-408 \\ -412$

 Table 1. Pumping data and field measurements.

Depths referenced to casing top. "n.d." = "not determined."

Table 2. Chemical composition of water samples.

Sample ID	$\sum CO_2$	$\sum Na$	$\sum Ca$	$\sum Mg$	$\sum Si$	NO_3^-	SO_4^{2-}	Cl-
BA1A_2018_55-66 BA1A_2018_100-400	1.32×10^{3} 3.74×10^{1}	8.36×10^{2} 2.81×10^{3}	3.91×10^2 1.57×10^3	7.06×10^2 5.00×10^1	1.97×10^{2} 4.49×10^{1}	2.40×10^{2} 1.85×10^{2}	2.70×10^2 5.18×10^2	$\begin{array}{c} 1.27\times10^3\\ 4.65\times10^3\end{array}$
BA1A_2019_0-30 BA1A_2019_41-65	3.18×10^{3} 1.42×10^{3}	5.13×10^2 6.83×10^2	5.89×10^2 3.64×10^2	1.38×10^{3} 1.05×10^{3}	3.33×10^2 1.56×10^2	1.21×10^2 9.03×10^1	3.21×10^2 3.36×10^2	9.34×10^2 1.27×10^3
BA1A_2019_108-132 BA1D_2019_45-75	$< 2.82 \times 10^2$ n.d.	3.36×10^{3} 4.03×10^{3}	1.69×10^{3} 2.55×10^{3}	$\begin{array}{c} 1.02\times10^1\\ 2.20\times10^1\end{array}$	$\begin{array}{c} 2.13\times10^1\\ 8.51\end{array}$	$< 8.06 \times 10^{-1}$ $< 8.06 \times 10^{-1}$	4.67×10^2 9.46×10^2	5.96×10^{3} 8.51×10^{3}
BA1D_2019_102-132	n.d.	5.18×10^3	2.92×10^3	1.56	5.88	$< 8.06 \times 10^{-1}$	$5.91 imes 10^2$	6.87×10^3

Concentrations reported in μ mol · L⁻¹. \sum indicates the sum of all dissolved species of the element. "n.d." = "not determined."

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2.3 Chemical and stable isotopic analyses of fluids

Chemical and stable isotopic analyses of fluids sampled in 2018 were conducted ac-154 cording to the methods reported by Nothaft et al. (2020). Analytical methods for flu-155 ids sampled in 2019 were similar and are reported below. To analyze aqueous concen-156 trations (c) of non-carbonaceous chemical species, samples were collected by passing ground-157 water through a $0.2 \,\mu m$ filter into polypropylene conical tubes. Solutes that were read-158 ily positively ionizable were measured by inductively coupled plasma atomic emission 159 spectroscopy (repeatability as median relative standard deviation of 3 % for most ele-160 ments). Aqueous concentrations of F^- Cl⁻, SO₄²⁻, Br⁻, and NO₃⁻ were measured by ion 161 chromatography (analytical uncertainty of 5%). Molybdate-reactive SiO₂ (reported here 162 as \sum Si) was quantitated using a spectrophotometric method (ASTM, 2016) (uncertainty 163 of 2% of measured value). The concentration and δ^{13} C of dissolved inorganic C ($\sum CO_2$) 164 were measured by acidification of water samples and transfer of resultant CO_2 (g) via 165 a Thermo Fisher GasBench II to a Thermo Delta V Plus isotope ratio mass spectrom-166 eter. Details of $\sum CO_2$ analyses are available at http://dx.doi.org/10.17504/protocols 167 .io.zduf26w. 168

Groundwaters and gases dissolved therein were sampled via syringe from a luerlok port on the pump manifold. 60. mL of this water was passed through a $0.2 \,\mu$ m filter and needle into an evacuated 117 mL glass vial capped with a blue chlorobutyl rubber stopper and Al crimp top. These are referred to as "headspace" samples. In addition, gases were sampled with the "bubble strip" method modified from Kampbell et al.

(1998). Details on bubble strip gas sampling are available at http://dx.doi.org/10.17504/ 174 protocols.io.bkb9ksr6. H_2 , CH_4 , and CO concentrations in this study are reported 175 from headspace samples due to the better accuracy of this method versus the bubble strip 176 method, as determined in comparisons by the authors. Ethane through hexane concen-177 trations were determined from the bubble strip samples because of the lower detection 178 limit offered by bubble strip samples, and were normalized to the headspace gas sam-179 ples through the CH_4 concentration and assumption of constant C_1/C_n ratio of both sam-180 ple types, where C_n is an alkane of n C atoms. Gas concentrations were determined ac-181 cording to the methods of Nothaft et al. (2020). 182

Bulk stable isotope analyses of CH_4 and co-occurring alkane gases were conducted 183 at the University of Colorado - Boulder (CUB) by GC/C/Pyr/IRMS using a Trace 1310 184 GC equipped with an Agilent J & W GS-CarbonPLOT column (30 m length, 0.32 mm 185 ID, $3.0 \,\mu\text{m}$ film) coupled to a Thermo Scientific MAT253 IRMS. Three CH₄ isotope stan-186 dards purchased from Airgas (uncertainties of ± 0.3 % for δ^{13} C and ± 5 % for δ D) and 187 three additional standards obtained from the U.S. Geological Survey (uncertainties of 188 $\pm 0.2\%$ for δ^{13} C and $\pm 3\%$ for δ D) were used for calibration. Over the range of peak am-189 plitudes of analyses reported here, the repeatability expressed as 1 s on analyses of stan-190 dards is $\pm 0.2 \%$ for δ^{13} C and $\pm 3 \%$ for δ D. The analytical uncertainty (accuracy) ex-191 pressed as 1 standard error on a 3-point calibration was 0.4% to 0.7% for $\delta^{13}C$ and 4%192 to 5 % for δD . 193

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2.4 16S rRNA gene sequencing and analysis

Biomass for DNA extraction was concentrated by pumping 5 L to 20 L of ground-195 water through sterile Millipore polycarbonate inline filters. In 2018, groundwaters were 196 passed sequentially through filters with pore diameters of $0.45 \,\mu\text{m}$, $0.22 \,\mu\text{m}$, then $0.10 \,\mu\text{m}$ 197 to test whether cell size and microbial community composition were correlated. In 2019, 198 only $0.22 \,\mu \text{m}$ pore diameter filters were used. The diameter of filters was 47 mm in both 199 years. Filters were placed in cryovials and stored for transport in liquid N_2 dewars im-200 mediately following biomass collection. Upon their arrival at CUB, filters were stored 201 in a $-70 \,^{\circ}$ C freezer until extraction. 202

For samples collected in 2018, DNA was extracted from one quarter subsamples 203 of each filter using a Qiagen PowerSoil DNA extraction kit following manufacturer in-204 structions, with the substitution of a 30-second bead beating step using a FastPrep-24 205 (MP Bio) homogenizer (instead of a standard vortexer) to more aggressively lyze cells. 206 The V4 hypervariable region of the 16S rRNA gene was amplified by PCR in duplicate 207 reactions using the 515 (Parada) - 806R (Apprill) primer pair modified to include Illumina adapters and the appropriate error-correcting barcodes, as described previously (Nothaft 209 et al., 2020). Amplicons from duplicate reactions were pooled, cleaned, and their con-210 centrations normalized using a Thermo Fisher SequalPrep normalization plate kit. Am-211 plicons were sequenced on an Illumina MiSeq at the CUB Next-Generation Sequencing 212 Facility using 2-by-150 bp paired-end chemistry. 213

For samples collected in 2019, DNA was extracted using the same methods as for 214 2018 samples. The V4 hypervariable region of the 16S SSU rRNA gene was amplified 215 216 from purified DNA by PCR. PCR was performed in triplicate reactions using the 515 (Parada) - 806R (Apprill) primer pair at an annealing temperature of 50C, as described 217 previously (Hamilton et al., 2013). Amplicons from triplicate reactions were pooled, and 218 adapters were added in triplicate reactions via five cycles of nested PCR (following the 219 same conditions as above) with 515 (Parada) - 806R (Apprill) primers modified to in-220 clude Illumina adapters. Amplicons from triplicate reactions were pooled and purified 221 using the Wizard PCR Preps DNA Purification System (Promega Corp.). Amplicons 222 were sequenced on an Illumina Miseq at the UW-Madison Biotechnology Center DNA 223 Sequencing Facility using 2-by-150 bp paired-end chemistry. 224

Demultiplexed fastq files were quality filtered using Figaro v1.1.1 (https://github .com/Zymo-Research/figaro) and the DADA2 v1.16 R package (Callahan et al., 2016). Amplicon sequence variants were assigned taxonomy to the genus level using the RDP classifier (Q. Wang et al., 2007) trained on the Silva SSU 138 reference database (Quast et al., 2012) using the DADA2 assignTaxonomy function. Sequences assigned to mitochondria, chloroplast, and Eukaryota, or not assigned at the domain level (collectively <1% of sequences), were removed.

²³² **3** Prior study of site hydrology

To provide context for the geochemical results that will follow, we summarize here 233 the findings of Lods et al. (2020), who interpreted flowmeter and pumping test data to 234 understand the physical hydrology of boreholes BA1A and BA1D. The present study's 235 samples from 2019 (Table 1) were collected simultaneously with, or immediately follow-236 ing, the pumping tests of Lods et al. (2020). Pumping depth intervals targeted hydrauli-237 cally conductive zones in the subsurface, which were inferred from temperature profiles 238 and flowmeter data (Lods et al., 2020). All hydraulically conductive regions were above 239 the transition from dunite to harzburgite, which occurs at depths of 160 m in BA1A and 240 250 m in BA1D (Figure 1; (P. Kelemen et al., 2020)). 241

Flowmeter tests under ambient and forced hydraulic conditions indicated the pres-242 ence of an aquifer in the highly weathered dunite bedrock at the contact between allu-243 vium and bedrock immediately below the casing at 22 m to 25 m in BA1A and 26 m to 244 27 m in BA1D. The transmissivity in the shallow regions of BA1A was higher than in 245 BA1D, and an ambient downflow of $1 \text{ L} \cdot \text{min}^{-1}$ measured in BA1A from 22 m to 59 m 246 indicated the displacement of substantial volumes of water from the surficial highly weath-247 ered dunites aquifer to lower dunite aquifers at BA1A at least as deep as 59 m. Lesser 248 flow may extend to even deeper aquifers at BA1A at rates below the detection limit of 249 the flowmeter used in the experiments of Lods et al. (2020) ($< 0.1 \,\mathrm{L} \cdot \mathrm{min}^{-1}$). Ambi-250 ent flow was below detectable levels at BA1D, suggesting minimal flow of surficial aquifer 251 waters to deeper aquifers at BA1D. 252

In conductive fractures between 41 m and 75 m depth, tests of pumping in BA1A indicated channelized, 1-dimensional flow between BA1A and BA1D. This flow was interpreted as passing through an open or partially mineralized fracture connected to the boreholes directly or through a conduit. All the pumped flow from BA1A in that interval could be accommodated through this channel. However, during pumping of BA1D in that interval, additional vertical flow from the formation near BA1D above and below the pumped interval was required to accommodate the pumped flow.

In contrast to the 41 m to 75 m aquifer, an aquifer between 102 m and 132 m dis-260 played no evidence of conductive structures with channelized flow. Rather, heterogeneities 261 in the directions of flow contributing to the pumping tests were inferred. The pumping 262 in BA1A was supplied by both horizontal and vertical flows that were both near and far 263 from the pumped borehole. Pumping in BA1D was also supplied by horizontal and ver-264 tical flows near the pumped borehole, but only horizontal flows further away, near BA1A. 265 Lods et al. (2020) speculated that a component of the water pumped during the BA1D 266 test could have been derived from the highly conductive fractures in the shallower re-267 gions near BA1A, with this water flowing downward through the BA1A borehole and 268 then horizontally to BA1D via fractures in the 102 m and 132 m depth interval. 269

Below 133 m, BA1D had sufficient transmissivity to accomodate pumping, but samples were not obtained from this interval for the present study due to low sustainable flow rates $(0.5 \text{ L} \cdot \text{min}^{-1})$. Vertical connections around BA1D link depths below 133 m to the 102 m and 132 m conductive interval of BA1D (and then to BA1A via horizontal connections). Vertical connections surrounding BA1A below 133 m were compara-

tively weaker, preventing successful pumping from this region of BA1A.

276 4 Results

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4.1 Aquifer geochemistry: drilling, mixing, and recovery

During drilling, a strong smell of sulfide was evident within tens of meters of BA1A, 278 suggesting sulfidic conditions in the subsurface at the time of drilling. In well logs ac-279 quired shortly after drilling of BA1A in 2017 and BA1D in 2018, Eh values were 100 mV 280 to 200 mV throughout most of the depth profile (Figure 1), indicating the presence of 281 oxidized fluids that were likely introduced at depth through drilling. In well logs obtained 282 at BA1A in 2018, a year after the drilling of that well, the chemical state of the upper 283 200 m of the depth profile was essentially unchanged since 2017, but the lower 200 m of 284 the depth profile showed a pH increase of 0.4 (reaching a maximum pH of 10.15), a stark 285 Eh decrease of 800 mV (reaching a minimum of $-599 \,\mathrm{mV}$), and an electrical conductiv-286 ity increase of up to $0.3 \,\mathrm{mS \cdot cm^{-1}}$ (reaching a maximum of $1.544 \,\mathrm{mS \cdot cm^{-1}}$). These data 287 indicate at least partial recovery towards reduced, hyperalkaline conditions at depths > 288 200 m at BA1A from 2017 to 2018. Similarly, in BA1D well logs obtained in 2019, a year 289 after the drilling of that well, a pH increase of 0.5 (reaching a maximum of pH of 11.05), 290 a stark Eh decrease of up to $700 \,\mathrm{mV}$ (reaching a minimum of $-623 \,\mathrm{mV}$), and an elec-291 trical conductivity increase of up to $0.8 \,\mathrm{mS \cdot cm^{-1}}$ (reaching $2.21 \,\mathrm{mS \cdot cm^{-1}}$) relative to the 292 2018 log were observed throughout most of the depth profile. A notable difference be-293 tween BA1A and BA1D well logs recorded a year after drilling is that the conditions in 294 BA1A transition from moderately alkaline and oxidized to hyperalkaline and reduced at $\sim 150 \,\mathrm{m}$ depth, while in BA1D, hyperalkaline and reduced conditions are reached at rel-296 atively shallow depths (40 m) and maintained to the bottom of the well (Figure 1). 297

The Eh, pH, and electrical conductivity in about half of the samples pumped from 298 discrete intervals using packers are similar to values at the same depth in the well logs 299 obtained a year after drilling. The other half of the samples have Eh, pH, and/or con-300 ductivity that are different from well log values at the same depth. For instance, the packer 301 samples BA1A_2018_100-400 and BA1A_2019_108-132 had higher pH values than in the 302 downhole log at any depth, and show pH > 10 at a much shallower depth than in the 303 2018 log, where such high pH is only reached below 200 m. At BA1D, pH values of pumped samples from 2019 overlap with those of well logs measured in the same year and at equiv-305 alent intervals, but the pumped samples had Eh values 200 mV lower and electrical con-306 ductivity values $0.5 \,\mathrm{mS} \cdot \mathrm{cm}^{-1}$ lower than the well logs at the same depths. 307

As expected based on pH, Eh, and electrical conductivity (Figure 1, Table 1), the 308 pH 8.1 to 8.7 waters in the upper 70 m of BA1A are $Mg^{2+}-HCO_3^-$ waters. The pH 10.6 309 to 11.0 waters at both sampled depths in BA1D and in the ≥ 100 m-depth samples from 310 BA1A are $Ca^{2+} - OH^-$ waters (Table 2). These are the two commonly observed water end-members in ophiolite aquifers, where $Mg^{2+} - HCO_3^-$ waters are considered to 311 312 communicate openly with the atmosphere and have shorter residence times, while Ca^{2+} -313 OH⁻ waters have extensively reacted with peridotite in regions of the subsurface closed 314 to atmospheric inputs (Barnes et al., 1967; Barnes & O'Neil, 1969; Neal & Stanger, 1985; 315 Bruni et al., 2002; Cipolli et al., 2004; P. B. Kelemen & Matter, 2008; P. B. Kelemen 316 et al., 2011; A. N. Paukert et al., 2012; Chavagnac, Monnin, et al., 2013; Boulart et al., 317 2013; Canovas III et al., 2017; Leong & Shock, 2020). These water types have distinct 318 \sum Si concentrations, as mineral dissolution in waters open to the atmosphere increases \sum Si in Mg²⁺ – HCO₃⁻ waters to 10² μ mol · L⁻¹ concentrations, while reaction under closed system, lower water/rock conditions in Ca²⁺–OH⁻ waters approaches chrysotile-319 320 321 brucite-calcite-diopside equilibrium and draws $\sum Si$ to $\mu mol \cdot L^{-1}$ to $10^2 n mol \cdot L^{-1}$ con-322 centrations (Leong et al., 2020). Leong et al. (2020) proposed the use of \sum Si as a tracer 323 of mixing in ophiolitic groundwaters, noting its relatively conservative behavior in these 324

- systems and its stronger sensitivity to mixing relative to pH. Adopting this approach,
- we have plotted the $c_{\sum \text{Si}}$ and pH of our samples in Figure 2. The shallowest sample in
- this study, BA1A_2019_0-30, had the highest $c_{\sum \text{Si}}$ of the data set (333 μ mol·L⁻¹), which
- is typical of $Mg^{2+}-HCO_3^-$ waters. Other samples fall below this, but do not reach the
- low levels representative of chrysotile-brucite-calcitediopside equilibrium as in end-member
- $Ca^{2+}-OH^{-}$ waters, suggesting that they represent mixtures of varying proportions of
- $Mg^{2+} HCO_3^-$ and $Ca^{2+} OH^-$ end-member waters.



Figure 2. \sum Si vs. pH plot, after Leong et al. (2020). The thick blue line corresponds to the reaction path model of Leong et al. (2020) starting from rain, progressing to moderately alkaline Mg^{2+} – HCO_3 waters as a response to mineral dissolution open to the atmosphere, then intermediate waters in early stages of serpentinization closed to the atmosphere, which later become hyperalkaline $Ca^{2+} - OH^-$ waters as they approach chrysotile-brucite-calcitediopside equilibrium. Three potential end member Ca^{2+} – OH^- water compositions (differing in their $c_{\sum CO_2}$ from $8 \,\mu$ mol \cdot kg⁻¹ to $20 \,\mu$ mol \cdot kg⁻¹) form one side of a mixing trend to a typical Mg²⁺ – HCO₃ water composition. Si, which is the most conservative tracer of mixing available in ophiolites, is used to distinguish extents of mixing between Ca^{2+} – OH^- and Mg^{2+} – HCO₃ waters (shown in plot as percentages next to mixing tie-lines). Mixing extents calculated for our samples are tabulated in Table S1. Other Samail Ophiolite data from (Neal & Stanger, 1985; Chavagnac, Monnin, et al., 2013; Miller et al., 2016; Rempfert et al., 2017; Canovas III et al., 2017; ?, ?; Nothaft et al., 2020; Paukert Vankeuren et al., 2019; Leong et al., 2020)

In the $Mg^{2+}-HCO_3^-$ waters in the upper 70 m of BA1A, dissolved inorganic carbon ($\sum CO_2$) ranged in concentration (c) from 1320 μ mol·L⁻¹ to 3180 μ mol·L⁻¹, similar to $c_{\sum CO_2}$ in other $Mg^{2+}-HCO_3^-$ waters in the ophiolite (Neal & Stanger, 1985; Chavagnac, Monnin, et al., 2013; Rempfert et al., 2017; Canovas III et al., 2017; Paukert Vankeuren et al., 2019; Nothaft et al., 2020; Leong et al., 2020), and consistent with uptake of atmospheric CO₂ into these moderately alkaline waters (Bruni et al., 2002; Cipolli et al., 2004; A. N. Paukert et al., 2012; Leong & Shock, 2020). In the Ca²⁺-OH⁻ waters sampled at \geq 100 m depths at BA1A, $c_{\sum CO_2}$ was 37.4 μ mol·L⁻¹ in 2018 and be-

low the limit of quantitation in 2019 ($< 282 \,\mu \text{mol} \cdot \text{L}^{-1}$; Table 2). Hyperalkaline ground-340 waters sampled from certain other wells in 2018, including the nearby (within 2 km), pH 341 \sim 11.4 well, NSHQ14, for which $c_{\sum \rm CO_2}$ was measured in the same analytical session 342 as BA1A_2018_100-400, had $c_{\sum CO_2}$ below the limit of quantitation in 2018 (< 12 μ mol· 343 L^{-1} ; (Nothaft et al., 2020)).¹ These lower values are consistent with water-harzburgite 344 reaction path modeling that terminates at chrysotile-brucite-diopside-calcite equilibrium, 345 corresponding to a $c_{\sum CO_2}$ of $8 \,\mu$ mol·L⁻¹ at 25 °C and 1 bar (Leong & Shock, 2020). Thus, the relatively higher $c_{\sum CO_2}$ in BA1A_2018_100-400 suggests that this sample does 346 347 not represent $Ca^{2+}-OH^-$ end-member water, but rather is the product of groundwa-348 ter mixing or moderate extents of water-rock reaction. Though not on the compositional 349 extremes of peridotite-hosted groundwaters, $\sum CO_2$ concentrations in the tens of μ mol·L⁻¹ or less in the Ca²⁺-OH⁻ waters of BA1A and BA1D are still quite low compared to seawater at 2.1 mmol·L⁻¹ or river water at 50 μ mol·L⁻¹ to 500 μ mol·L⁻¹ (Zeebe 350 351 352 & Wolf-Gladrow, 2001; Waldron et al., 2007). 353

Concentrations of the reduced gases H_2 and CH_4 ranged up to $4.05 \,\mu \text{mol} \cdot \text{L}^{-1}$ and $3.81 \,\mu \text{mol} \cdot \text{L}^{-1}$, respectively, in $\text{Ca}^{2+} - \text{OH}^-$ waters of BA1A and BA1D, whereas H_2 354 355 and CH_4 were below limits of quantitation in the $Mg^{2+} - HCO_3^-$ waters from the up-356 per 70 m of BA1A (Table 3), consistent with the differing Eh of these waters (Table 1). 357 The concentrations of H_2 and CH_4 in the $Ca^{2+} - OH^-$ waters are high in comparison 358 to near-surface aquifers in sedimentary settings, where H_2 concentrations rarely exceed 359 $10 \text{ nmol} \cdot \text{L}^{-1}$, even under the most reduced conditions (Lovley et al., 1994; Kampbell 360 et al., 1998), but they are moderate in the context of peridotite aquifers, as in the Samail 361 Ophiolite, where groundwaters accessed at wells can have H₂ and CH₄ concentrations 362 in the hundreds to thousands of μ mol·L⁻¹ (A. Paukert, 2014; Nothaft et al., 2020). In addition, dissolved $C_2 - C_6$ alkanes were detected in some samples (Table 3). In sam-364 ples with quantitatable C_2H_6 , CH_4/C_2H_6 ratios ranged from 14.6 to 106, lower than $CH_4/(C_2H_6 \pm C_3H_8)$ 365 ratios of 10^2 to 10^4 previously reported in other samples from the Samail Ophiolite (Fig-366 ure S3; (Etiope et al., 2015; Vacquand et al., 2018; Nothaft et al., 2020)). 367

To assess the availability of oxyanions as terminal electron acceptors for microbial 368 metabolism, NO_3^- and SO_4^{2-} concentrations were measured (Table 2). In samples of BA1A 369 taken in 2018, NO_3^- concentrations were higher in samples from depths of 55 m to 66 m 370 $(240. \mu \text{mol} \cdot \text{L}^{-1})$ than in samples from depths of 100 m to $400 \text{ m} (185 \mu \text{mol} \cdot \text{L}^{-1})$. In 371 samples of BA1A taken in 2019, a trend of decreasing $c_{\rm NO_3^-}$ with increasing depth was also observed, with samples from depths of 0 m to 30 m, 41 m to 65 m, and 108 m to 132 m 372 373 having NO₃⁻ concentrations of $132 \,\mu \text{mol} \cdot \text{L}^{-1}$, $90.3 \,\mu \text{mol} \cdot \text{L}^{-1}$, and below the limit of 374 quantitation (< $0.806 \,\mu \text{mol} \cdot \text{L}^{-1}$), respectively. The higher NO₃⁻ concentrations are within 375 the range previously reported for $Mg^{2+}-HCO_3^-$ waters sampled from wells in the ophi-376 olite (Rempfert et al., 2017; Nothaft et al., 2020). NO_3^- was below the limit of quanti-377 tation in all samples from BA1D. Thus, concentrations of NO_3^- were higher in more ox-378 idized aquifers at BA1A and BA1D (Table 1; Figure 1). 379

In contrast, SO_4^{2-} concentrations were generally higher in more reduced and hyperalkaline water samples (Table 2; Figure S5), reaching 946 μ mol·L⁻¹ in BA1D_2019_45-75. The higher SO_4^{2-} concentrations in BA1A and BA1D are higher than in other Ca²⁺ – OH⁻ waters in the Samail Ophiolite, in which SO_4^{2-} concentrations are usually in the tens of μ mol·L⁻¹ and rarely exceed 500 μ mol·L⁻¹ (Figure S5). Compared to Ca²⁺ – OH⁻ waters, SO_4^{2-} concentrations are typically higher in Mg²⁺-HCO₃⁻ waters (100 μ mol·

¹ The limit of quantitation for $c_{\sum CO_2}$ was higher in 2019 than in 2018 because a set of higher volume samples from 2019 were compromised during transport. We usually take two different sample volumes to ensure that, upon acidification, both Mg²⁺ – HCO₃⁻ and Ca²⁺ – OH⁻ end-member waters have optimal m/z 44 signal intensity for mass spectrometer analysis.

Sample ID	${\rm H}_2$	CO	CH_4	C_2H_6	$C_{3}H_{8}$	i-C ₄ H ₁₀	n -C $_4$ H $_{10}$	i-C ₅ H ₁₂	n-C ₅ H ₁₂	$\mathrm{C_6H_{14}{}^a}$
BA1A_2018_55-66	$< 5.98 \times 10^{-1}$	$< 1.32 \times 10^{-1}$	$< 1.18 \times 10^{-1}$	$< 9.88 \times 10^{-4}$	$< 7.60 imes 10^{-4}$	$< 4.61 \times 10^{-4}$	$<5.78\times10^{-4}$	$< 3.43 \times 10^{-4}$	$< 3.81 imes 10^{-4}$	$< 2.81 imes 10^{-4}$
BA1A_2018_100-400	4.05	$< 1.32 \times 10^{-1}$	2.42×10^{-1}	$< 9.88 \times 10^{-4}$	$< 7.60 \times 10^{-4}$	$< 4.61 \times 10^{-4}$	$3.89 imes 10^{-2}$	$< 3.43 \times 10^{-4}$	$< 3.81 \times 10^{-4}$	$8.80 imes 10^{-4}$
$BA1A_{2019_{-}0-30}$	< 3.29	$< 2.04 \times 10^{-2}$	$< 6.70 \times 10^{-1}$	$< 8.63 \times 10^{-3}$	$< 6.81 \times 10^{-3}$	$< 4.13 \times 10^{-3}$	$< 5.45 \times 10^{-3}$	$< 3.27 imes 10^{-3}$	$< 3.63 \times 10^{-3}$	$< 2.77 imes 10^{-3}$
$BA1A_{2019}41-65$	< 3.29	$< 2.04 \times 10^{-2}$	$< 6.70 \times 10^{-1}$	$< 8.63 \times 10^{-3}$	$< 6.81 \times 10^{-3}$	$< 4.13 \times 10^{-3}$	$< 5.45 \times 10^{-3}$	$< 3.27 imes 10^{-3}$	$< 3.63 \times 10^{-3}$	$< 2.77 imes 10^{-3}$
BA1A_2019_108-132	< 3.29	$< 2.04 \times 10^{-2}$	1.05	$7.16 imes 10^{-2}$	$< 6.81 \times 10^{-3}$	$< 4.13 \times 10^{-3}$	$< 5.45 \times 10^{-3}$	$< 3.27 imes 10^{-3}$	$< 3.63 \times 10^{-3}$	$< 2.77 imes 10^{-3}$
$BA1D_{-2019_{-}45_{-}75}$	< 3.29	$< 2.04 \times 10^{-2}$	2.30	$2.16 imes 10^{-2}$	$< 6.81 \times 10^{-3}$	$< 4.13 \times 10^{-3}$	$< 5.45 \times 10^{-3}$	$< 3.27 imes 10^{-3}$	$< 3.63 \times 10^{-3}$	$< 2.77 imes 10^{-3}$
BA1D_2019_102-132	3.55	$< 2.04 \times 10^{-2}$	3.81	$1.27 imes 10^{-1}$	$< 6.81 \times 10^{-3}$	$<4.13\times10^{-3}$	$<5.45\times10^{-3}$	$< 3.27 imes 10^{-3}$	$< 3.63 \times 10^{-3}$	$< 2.77 imes 10^{-3}$
			^a Hexane	e isomers not	chromatograf	ohically resolv	ed.			

[ti]	Aqueous gas concentrations, reported in μ mol \cdot L ⁻¹ .
	Table 3.

³⁸⁶ L^{-1} to 1000 μ mol· L^{-1}) and gabbro waters (500 μ mol· L^{-1} to 4000 μ mol· L^{-1}) in the ³⁸⁷ Ophiolite (Figure S5). The co-existence of SO₄²⁻ approaching mmol· L^{-1} levels and H₂ ³⁸⁸ at μ mol· L^{-1} levels in some Ca²⁺ – OH⁻ waters at BA1A and BA1D could make mi-³⁹⁹ crobial SO₄²⁻ reduction coupled to H₂ oxidation a viable metabolic strategy in those wa-³⁹⁰ ters.

391

4.2 Stable isotopic compositions of water, $\sum CO_2$, CH₄ and C₂H₆

To trace H and C through the BA1 system, the stable isotopic compositions of wa-392 ter, $\sum CO_2$, CH₄, and C₂H₆ were measured. Groundwater δD and $\delta^{18}O$ data plot near 393 local and global meteoric water lines (Weyhenmeyer et al., 2002; Terzer et al., 2013), in-394 dicating that the groundwaters are derived from rain (Table 4; Figure S4; (Matter et al., 395 2006; Miller et al., 2016; Paukert Vankeuren et al., 2019; Nothaft et al., 2020)). The $\delta^{13}C_{\sum CO_2}$ 396 of Mg²⁺-HCO₃⁻ waters in the upper 70 m of BA1A ranged from -14.64 % VPDB to -14.15 % VPDB (Table 4), which is within the range of $\delta^{13}C_{\sum CO_2}$ of Mg²⁺-HCO₃⁻ waters elsewhere in the ophiolite (-15.56 % VPDB to -10.88 % VPDB; (Matter et al., 397 398 399 2006; Nothaft et al., 2020)). These values are considerably lower than seawater $\delta^{13}C_{\sum CO_2}$, 400 which ranges from 0 % VPDB to 2 % VPDB (Zeebe & Wolf-Gladrow, 2001). This difference is peculiar because, like seawater, $Mg^{2+}-HCO_3^-$ waters in ophiolites have HCO_3^- 401 402 as the dominant $\sum CO_2$ species and are widely thought to be close to equilibrium with 403 atmospheric CO₂ (Neal & Stanger, 1985; Bruni et al., 2002; Cipolli et al., 2004; A. N. Pauk-404 ert et al., 2012; Leong & Shock, 2020). In comparison to the $Mg^{2+} - HCO_3^-$ water at 405 BA1A, a deeper sample (BA1A_2018_100-400) bearing $Ca^{2+}-OH^-$ water had notably lower $\delta^{13}C_{\sum CO_2}$ (-18.0 % VPDB; Table 4). The dynamics of $\delta^{13}C_{\sum CO_2}$ in this sys-406 407 tem will be discussed further in Section 5.4. 408

Sample ID	$\delta D_{\rm H_2O}$	$\delta^{18} \mathrm{O}_{\mathrm{H_2O}}$	$\delta^{13}C_{\sum CO_2}$	$\delta^{13} \mathrm{C}_{\mathrm{CH}_4}$	$\delta D_{\rm CH_4}$	$\delta^{13}C_{C_2H_6}$
BA1A_2018_55-66 BA1A_2018_100-400 BA1A_2019_0-30 BA1A_2019_41-65 BA1A_2019_108-132	-10.9 -9.4 -10.8 -9.77 -3.92	$-2.55 \\ -2.17 \\ -2.44 \\ -2.27 \\ -0.91 \\ 1.24$	$-14.64 \\ -18.0 \\ -14.15 \\ -14.32 \\ \text{n.d.}$	n.d. n.d. n.d. 23.9	n.d. n.d. n.d. 45	n.d. n.d. n.d. n.d. n.d.
BA1D_2019_45-75 BA1D_2019_102-132	$-4.52 \\ -6.9$	$-1.04 \\ -1.59$	n.d. n.d.	$\frac{12.8}{3.8}$	$-111 \\ -112$	n.d. -2.5

Table 4. Stable isotopic compositions of water, $\sum CO_2$, CH_4 and C_2H_6 .

All δ values reported in % units. δ^{18} O and δ D reported relative to VSMOW. δ^{13} C reported relative to VPDB. "n.d." = "not determined."

 $_{409}$ Three samples had sufficient CH₄ for accurate isotopic analysis using our methods

(Table 4). BA1D_2019_102-132 had a $\delta^{13}C_{CH_4}$ of 3.8 % VPDB, which is high compared

 $_{411}$ to CH₄ typically found in sedimentary settings, but within the range of CH₄ in serpen-

- tinizing settings, including the Samail Ophiolite (Figure 3; (Milkov & Etiope, 2018)). BA1D_2019_45-
- ⁴¹³ 75 had a $\delta^{13}C_{CH_4}$ of 12.8 % VPDB, which is higher than previously reported for CH₄
- in Samail Ophiolite, and BA1A_2019_108-132 had even higher $\delta^{13}C_{CH_4}$ (23.9% VPDB).



Figure 3. Plot of δD_{CH_4} vs. $\delta^{13}C_{CH_4}$ of samples from BA1A and BA1D with previously published Samail Ophiolite samples (Etiope et al., 2015; Vacquand et al., 2018; Nothaft et al., 2020) shown in black for context. Shaded fields of typical gas origin after Milkov and Etiope (2018). *Abbreviations:* PM, primary microbial; SM, secondary microbial; T, thermogenic; A, abiotic.

In addition to CH_4 , there was sufficient C_2H_6 for isotopic analysis in sample BA1D_2019_102-415 132, and this C_2H_6 was also ¹³C-enriched (-2.5 % VPDB; Table 4) compared to C_2H_6 416 typically found in sedimentary settings (Prinzhofer & Huc, 1995). However, this $\delta^{13}C_{C_2H_6}$ 417 value is generally similar to that previously reported for C_2H_6 in the Samail Ophiolite 418 (Figure 4; (Fritz et al., 1992; Nothaft et al., 2020)), suggesting an abiotic source of C_2H_6 419 at BA1D. The $\delta^{13}C_{C_2H_6}$ of BA1D_2019_102-132 is notably similar (within 3.5 %) to $\delta^{13}C_{C_2H_6}$ 420 of well NSHQ14 (Nothaft et al., 2020), which is only 2 km down-gradient within the same 421 catchment, suggesting a similar source of C_2H_6 in these wells. 422



Figure 4. Plot of δ^{13} C of CH₄ and co-occurring *n*-alkanes vs. the number of C atoms per molecule. Error bars represent uncertainties on δ^{13} C analyses performed at CUB. Only samples for which δ^{13} C_{C2} was determined are plotted. Contextual data from the Samail Ophiolite at Nizwa (Fritz et al., 1992) and well NSHQ14 (Nothaft et al., 2020).

The δD_{CH_4} at BA1D (-111 % VSMOW to -112 % VSMOW; Table 4) is higher 423 than that of samples previously reported from the Samail Ophiolite (Figure 3), but sim-424 ilar to that of samples from sediment-poor seafloor hydrothermal vents that are near iso-425 topic equilibrium with ocean water at 270 °C to 360 °C (D. T. Wang et al., 2018; Labidi 426 et al., 2020) and similar to some samples from ophiolites, including those in the Philip-427 pines (Abrajano et al., 1990) and Turkey (Young et al., 2017), where dominantly abi-428 otic sources of CH₄ have been proposed. Thus, it is plausible, from the perspective of 429 δD_{CH_4} , that BA1D CH₄ formed abiotically and equilibrated, potentially at 270 °C to 360 °C, 430 with water with δD similar to that of seawater. The δD_{CH_4} of BA1A_2019_108-132, how-431 ever, is extraordinarily high (45 % VSMOW). Noting that the δD of water at BA1A, BA1D, 432 and other wells in the Samail Ophiolite is within 15 % of VSMOW (Table 2; (Miller et 433 al., 2016; Paukert Vankeuren et al., 2019; Nothaft et al., 2020)), the high δD of CH₄ from 434 $BA1A_2019_{-108-132}$ cannot plausibly be explained by CH_4 having equilibrated with wa-435 ter (Horibe & Craig, 1995). Moreover, it is unlikely to have been produced through ki-436 netic processes, in which CH_4 would be expected to be D-depleted with respect to the 437 H of its precursor. Thus, the δD_{CH_4} of BA1A_2019_108-132 likely indicates post-genetic 438 fractionation, perhaps during microbial CH_4 oxidation. CH_4 oxidation is also compat-439 ible with the low $CH_4/(C_2H_6 + C_3H_8)$ in BA1A and BA1D samples, particularly BA1A_2019_108-440 132 ($CH_4/C_2H_6 = 14.6$), relative to other samples from the Samail Ophiolite (Figure 441 S3). 442

443

4.3 16S rRNA gene sequencing

To assess microbial community composition, 16S rRNA genes of DNA extracted 444 from biomass that was concentrated from groundwaters were amplified and sequenced. 445 16S rRNA gene reads affiliated with class Thermodesulfovibrionia were dominant in the 446 BA1A and BA1D data set, accounting for more than 90% of reads in some samples (Fig-447 ure 5), particularly those with low Eh (Table 1). Cultured representatives of Thermod-448 esulfovibrionia are capable of SO_4^{2-} reduction coupled to H_2 oxidation and may addi-449 tionally/alternatively oxidize C_1 - C_3 acids and use thiosulfate, sulfite, Fe^{3+} or NO_3^- as 450 terminal electron acceptors for anaerobic respiration (Henry et al., 1994; Sekiguchi et al., 451 2008; Frank et al., 2016). The high relative abundance of Thermodesulfovibrionia, es-452

⁴⁵³ pecially in samples that are reduced, contain up to $\mu \text{mol} \cdot \text{L}^{-1}$ levels of H₂, approach ⁴⁵⁴ mmol·L⁻¹ levels of SO₄²⁻, and have below quantitatable (sub- $\mu \text{mol·L}^{-1}$) levels of NO₃⁻, ⁴⁵⁵ suggests that microbial sulfate reduction may be an important process in the subsurface ⁴⁵⁶ at BA1A and BA1D.

Taxa that had markedly higher relative abundances of 16S rRNA genes in the more 457 oxidized waters sampled from the upper 70 m of BA1A include relatives of the genus Brachy-458 monas and the species Parvibaculum lavamentivorans (Figure 5). Cultured representa-459 tives of *Brachymonas* are heterotrophic and respire using molecular oxygen, and in some 460 cases nitrate, as terminal electron acceptors (Hiraishi et al., 1995; Halpern et al., 2009). 461 A close relative of *Brachymonas denitrificans* was enriched with H_2 and formate using 462 groundwater sampled from wells in the Coast Range Ophiolite, USA as innoculum (Crespo-463 Medina et al., 2014). This indicates that *Brachymonas* relatives can thrive in anaero-464 bic conditions in serpentinization-derived fluids. Parvibaculum lavamentivorans isolates 465 are aerobic heterotrophs (Schleheck et al., 2011), but *Parvibaculum* species have also been 466 detected through culture-independent methods in enrichment cultures under anaerobic, 467 denitrifying conditions (Blöthe & Roden, 2009; De Weert et al., 2011). The high rela-468 tive abundances of Brachymonas- and P. lavamentivorans-affiliated 16S rRNA gene reads 469 in the more oxidized parts of BA1A, taken together with the trends of decreasing $c_{\rm NO_{-}}$ 470 with increasing depth at BA1A (Section 4.1), suggest that heterotrophic, aerobic and/or 471 denitrifying microbial metabolisms may be active in those regions of the subsurface. 472

Taxa that also had high 16S rRNA gene relative abundances in some samples, but 473 whose metabolic functions are more enigmatic, include relatives of the genus *Meiother*-474 mus. 16S rRNA gene reads affiliated with Meiothermus were detected in all BA1A and 475 BA1D samples, but had the highest relative abundance (22% of reads) in the sample BA1A_2018_100-476 400 (Figure 5). Although Eh was not directly measured for BA1A_2018_100-400, this sample had $4.05 \,\mu\text{mol}\cdot\text{L}^{-1}$ H₂ (Table 3) and a pH of 10.69 (Table 2), suggesting that 478 it was reduced, which would be consistent with a sample from a similar depth interval 479 taken the following year (BA1A_2019_108-132), which had an Eh of $-249 \,\mathrm{mV}$ (Table 1). 480 Meiothermus has been a confounding taxon in 16S rRNA gene surveys of the Samail Ophi-481 olite subsurface because Meiothermus isolates reported in the literature have thus far 482 been shown to be aerobic (although some can reduce NO_3^- to NO_2^- ; (Habib et al., 2017; 483 Raposo et al., 2019)). Nonetheless, Meiothermus-affiliated 16S rRNA gene sequences have 484 consistently accounted for high percentages of reads from the most reduced (lowest f_{O_2}) 485 groundwater samples from wells in the Samail Ophiolite, where they apparently cohab-486 itate with obligate anaerobes (Miller et al., 2016; Rempfert et al., 2017; Nothaft et al., 487 2020; Kraus et al., 2021). Previous studies of the ophiolite have suggested that Meio-488 thermus could have been functioning anaerobically, or could have been inhabiting shal-489 low, oxic waters that were mixed with deeper, anoxic waters during open-borehole pump-490 ing (Miller et al., 2016; Rempfert et al., 2017). The present study's finding of high rel-491 ative abundances of *Meiothermus* 16S rRNA gene reads in a sample obtained through 492 pumping of $a > 100 \,\mathrm{m}$ depth interval isolated using packers favors the interpetation that 493 Meiothermus are indeed capable of functioning anaerobically in the subsurface. Culti-494 vation approaches or metagenomic inferences of the functionality of these organisms based 495 on gene complements will be required to determine how Meiothermus sp. persists in these 496 highly reduced waters. 497

Another enigmatic taxon is candidate phylum GAL15, which comprised 26 % of 498 16S rRNA gene reads in the sample BA1D_2019_102-132 (Figure 5). No genomes or cul-499 tured isolates from this taxon have been published, so its traits can only be inferred in-500 directly. The relative abundance of GAL15 in 16S rRNA gene amplicon sequences and 501 shotgun metagenomic sequences was found to positively correlate with increasing depth 502 in a study of 20 soil profiles in diverse ecological settings throughout the United States, 503 suggesting that members of GAL15 are well-suited to the oligotrophic conditions of rel-504 atively deep (1 m) soil horizons (Brewer et al., 2019). Members of GAL15 have also been 505

		2018 E	3A1A	2(019 BA1A		2019 B/	A1D
k_Bacteria; p_Nitrospirota; c_Thermodesulfovibrionia; o_NA; f	IA; f_NA; g_NA; s_NA -	20	33	15	43	06	92	63
k Bacteria; p Proteobacteria; c Gammaproteobacteria; o Burkholderiales; f Comamonadaceae; g B	g Brachymonas; s NA -	32	6	.	24	e	ř	ř
k Bacteria; p Proteobacteria; c Alphaproteobacteria; o Parvibaculales; f Parvibaculaceae; g Parvibaculum;	ulum; s lavamentivorans -	¥	¥	36	-	¥	Ŷ	Ŷ
k Bacteria; p Deinococcota; c Deinococci; o Thermales; f Thermaceae; g N	g Meiothermus; s NA-	5	22	2	٢	2	e S	v
k Bacteria; p GAL15; c NA; o NA; f	IA; f NA; g NA; s NA-	n.r.	n.r.	v	v	ř	ř	26
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_NA; f_	IA; f_NA; g_NA; s_NA-	4	4	v	5	v	n.r.	n.r.
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Sutterellac	erellaceae; g_NA; s_NA -	ř	-	7	2	v	n.r.	Ŷ
iso k_Archaea; p_Crenarchaeota; c_Nitrososphaeria; o_Nitrosopumilales; f_Nitrosopumilaceae; g_Candidatus_N	itus_Nitrosotenuis; s_NA -	2	-	-	5	v	n.r.	n.r.
k_Bacteria; p_Bacteroidota; c_lgnavibacteria; o_lgnavibacteriales; f_SM1	SM1H02; g_NA; s_NA-	n.r.	n.r.	7	-	n.r.	v	n.r.
k_Bacteria; p_NA; c_NA; o_NA; f	IA; f_NA; g_NA; s_NA-	2	ř	4	v	v	v	ř
6 K_Bacteria; p_Verrucomicrobiota; c_Verrucomicrobiae; o_Opitutales; f_Opitutaceae; g_La	I_Lacunisphaera; s_NA -	9	¥	v	v	n.r.	n.r.	n.r.
6 k_Archaea; p_Crenarchaeota; c_Nitrososphaeria; o_Nitrososphaerales; f_Nitrososphaeraee; g_Candidatus_Nitr	s_Nitrososphaera; s_NA -	с	v	-	-	n.r.	n.r.	n.r.
k Bacteria; p Proteobacteria; c Gammaproteobacteria; o Xanthomonadales; f Xanthomonadaceae; g	; g_Silanimonas; s_NA -	n.r.	-	2	v	-	ř	-
t k_Bacteria; p_Patescibacteria; c_CPR2; o_NA; f	IA; f_NA; g_NA; s_NA -	v	n.r.	4	v	n.r.	v	n.r.
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Xanthomonadales; f_Xanthomonadac	nadaceae; g_NA; s_NA -	-	-	v	e	v	n.r.	n.r.
Comparing the sector of the	Azohydromonas; s_NA-	v	e	n.r.	-	n.r.	n.r.	n.r.
k_Bacteria; p_Patescibacteria; c_Parcubacteria; o_NA; f	IA; f_NA; g_NA; s_NA -	2	v	v	v	n.r.	-	n.r.
k_Bacteria; p_Nitrospirota; c_Nitrospirai; o_Nitrospirales; f_Nitrospiraceae; g	ee; gNitrospira; s_NA -	-	v	2	-	v	n.r.	n.r.
k_Bacteria; p_Acetothermia; c_Acetothermia; o_NA; f	IA; f_NA; g_NA; s_NA-	n.r.	n.r.	v	v	n.r.	ř	7
k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Rhodocyclaceae; g_Meth	Methyloversatilis; s_NA -	v	٢					
	Other taxa -	22	23	16	10	4	з	7
	J	55-66	oth of sa	0-30 tmpling of samp	41-65 1 interval	08-132 / [m] gr d well na	45-75 10 buped b me	02-132 y
		Ϋ́β	ad relat	ive				
		an	ninalio	[0/] / 2	0	50	75	
Figure 5. 16S rRNA gene read relative abundances of 20 most abundant taxonomic assignment	nents in DNA extract	ed fro	n 0.22 /	ım-filte	r-conce	ntrated	eround	
waters from BA1A and BA1D . Read relative abundances are reported as percentages rounded to t	to the ones place. Ca	ases wł	, ien a ta	xon we	us detec	ted in a	sample	
and was $< 1\%$ read relative abundance after rounding are labeled " < 1 ". Cases when no rea	reads of a taxon were	e detec	ted in a	a sampl	le, but	when th	at taxo	n was
detected in 16S gene reads of other Oman samples obtained during the same sampling year, are lak	e labeled "n.r." Cases	when	no read	ls were	detecte	in an	7 Oman	

sample within the data set of a given year are blank.

detected in sediments at radionuclide-contaminated sites (Lin et al., 2012) and in high-506 altitude, cold fumarolic environments (Costello et al., 2009), suggesting that members 507 of GAL15 are stress-tolerant. Members of GAL15 were found to be more abundant in 508 oxic than anoxic zones of a profile of sediment cores (Lin et al., 2012), suggesting that 509 some members of GAL15 are tolerant of oxic conditions. However, our finding of a high 510 relative abundance of GAL15-affiliated 16S rRNA gene reads in a sample of strongly re-511 duced waters (-412 mV Eh and $3.55 \,\mu \text{mol} \cdot \text{L}^{-1}$ H₂; Tables 1 and 3) pumped from an 512 isolated depth interval of $102 \,\mathrm{m}$ to $132 \,\mathrm{m}$ in a peridotite aquifer suggests that some mem-513 bers of GAL15 are anaerobes. 514

16S rRNA gene sequences were searched for matches to known CH₄-cycling taxa, 515 as compiled previously (Crespo-Medina et al., 2017; Nothaft et al., 2020). Sequences closely 516 affiliated with both methanogenic and aerobic methanotrophic taxa were found in mul-517 tiple samples, but always in low relative abundance (< 1% of reads; Figure S6). These 518 included sequences related to the genus Methanobacterium, whose members can produce 519 CH_4 from H_2 and CO_2 , CO, or formate (Balch et al., 1979) and are widespread in Samail 520 Ophiolite groundwaters (Miller et al., 2016; Rempfert et al., 2017; Fones et al., 2019; Nothaft 521 et al., 2020; Fones et al., 2020). In addition, relatives of aerobic methanotrophs of the 522 genus Methylocaldum (Hanson & Hanson, 1996) and the family Methylacidiphilaceae (Op den 523 Camp et al., 2009) were detected. 524

Previous studies have shown that organisms inhabiting environments that impose 525 stress often exhibit smaller cells sizes (Luef et al., 2015). To explore whether this phe-526 nomenon is also true in the Samail Ophiolite, biomass was collected sequentially onto filters with pore diameters of $0.45 \,\mu\text{m}$, $0.22 \,\mu\text{m}$, and $0.10 \,\mu\text{m}$ by connecting three inline 528 filter housings in series when sampling BA1A in 2018 (Figure S7, Text S1, Figure S2). 529 Microbial community compositions were similar across filters of different pore diameters 530 (Figure S7), with the exception of relatives of the genus *Brachymonas*, which had high-531 est relative abundance in the $0.10 \,\mu\text{m}$ pore-diameter filter in the sample from $> 100 \,\text{m}$ 532 depth (80% of reads, compared to 9% to 10% in filters of other pore diameters), but 533 showed decreased relative read abundance with decreasing pore size in samples of the 534 55 m to 66 m depth interval (44 %, 32 %, and 26 % in the 0.45 μm , 0.22 μm , and 0.10 μm 535 pore-diameter filters, respectively). Although a greater sample size would be required 536 to robustly interpret trends of microbial community composition as a function of cell size 537 in this environment, our ability to extract and sequence DNA from cells between $0.22 \,\mu m$ 538 and $0.10 \,\mu\text{m}$ in diameter is in itself notable because streamlining (shrinking) of cell and/or 539 genome sizes has been proposed as an adaptive strategy to reduce the energetic costs of 540 replication under conditions of environmental stress (Giovannoni et al., 2014), includ-541 ing the challenging conditions of high pH and low electron acceptor and inorganic C avail-542 ability found in parts of the Samail Ophiolite (Suzuki et al., 2017; Fones et al., 2019). 543

In addition to biomass samples obtained during pumping, a sample of drill foam/fluid was taken as it emerged from BA1A during drilling in order to evaluate potential contamination of the subsurface with exogenous microbes. The most abundant 16S rRNA gene sequences in the drill foam/fluid sample were distinct from those of samples obtained during subsequent groundwater pumping (Figure S8, Text S1). This suggests that most of the taxa identified in samples subsequently obtained from pumping groundwaters from the subsurface were not derived from drill fluids.

551 5 Discussion

552

5.1 Sources and mixing of groundwaters

To derive an integrated hydrologic and geochemical conceptual model of the BA1A/BA1D system, we revisit the trends in the wireline logs and pumped sample geochemistry in light of the physical hydrological conclusions of Lods et al. (2020) (Section 3). In the case

of BA1A, where downward flow within the borehole under ambient conditions has been 556 recorded (Section 3), it appears that the wireline $\log pH$, Eh, and electrical conductiv-557 ity trends (Figure 1; Section 4.1) reflect displacement of moderately alkaline water from 558 the surficial $(< 27 \,\mathrm{m})$ aquifer down to depths approaching 200 m, where it mixes in the 559 borehole with hyperalkaline $Ca^{2+}-OH^{-}$ waters from deeper aquifers. In contrast, pumped 560 samples of packed intervals at depths $> 100 \,\mathrm{m}$ in BA1A drew water from the surround-561 ing rock formations that was more hyperalkaline and reduced (Table 1) than in the open 562 borehole at the same depth. In BA1D, where no ambient flow within the borehole was 563 recorded (Section 3), the well logs and pumped samples show closer agreement. Thus, 564 differences in logs and pumped sample chemical compositions in BA1A and BA1D may 565 result from variable within-borehole flow regimes. We speculate that, prior to drilling, 566 the 41 m to 75 m aquifer near BA1A may have been reduced and hyperalkaline, as BA1D 567 was in that depth interval during sampling in 2019, and that the strong drilling-induced 568 connection of the surficial (< 27 m) aquifer to the 41 m to 75 m aquifer through the BA1A 569 borehole resulted in the oxidized and moderately alkaline compositions recorded in our samples of BA1A at depths $< 70 \,\mathrm{m}$ (Table 1). 571

The \sum Si and pH data provide further insights into aquifer dynamics. Although 572 the aquifers are nominally in dunite, the pH of $Ca^{2+} - OH^-$ waters (≥ 10.67 ; Table 573 1) indicates that they must have reacted with diopside along their flow paths. Reaction 574 of water with Mg-endmember olivine (forsterite) alone will reach chrysotile-brucite equi-575 librium at pH ~ 10 (Leong & Shock, 2020). The addition of enstatite is also insufficient 576 to increase pH past 11. Rather, pH exceeds 11 only when diopside is present (Leong & 577 Shock, 2020). Thus, there may be some small amount of diopside within the dunites, or 578 the waters may have sourced from outside of the immediate, dunitic surroundings of the boreholes. Both are possible because small amounts of diopside are common in Oman 580 dunites (P. B. Kelemen et al., 1995), and harzburgites are present throughout the catch-581 ment where BA1A and BA1D are situated. 582

The indication that these waters have reacted with olivine and diopside validates 583 the use of the Leong et al. (2020) Si mixing model. Our samples plot close to the mix-584 ing line associated with the $20 \,\mu \text{mol} \,\text{kg}^{-1} \, c_{\sum \text{CO}_2}$ chrysotile-brucite-calcite end mem-585 ber of Leong et al. (2020) (leftmost dotted mixing line in Figure 2). Assuming this as a compositional end-member, the extent of mixing with a $Mg^{2+} - HCO_3^-$ water end-586 member is shown in Figure 2 and tabulated in Table S1. These calculations reveal a trend of decreasing contribution of $Mg^{2+}-HCO_3^-$ water to the total water mass with increas-588 589 ing depth in BA1A. This is consistent with the hydrologic flowmeter tests that showed 590 strong downflow in the upper 60 m at BA1 (Lods et al., 2020). At greater depths, am-591 bient flow was below detection (< $0.1 \,\mathrm{L} \cdot \mathrm{min}^{-1}$), but appears to slowly influence the 592 chemical composition of borehole waters to depths approaching 200 m, as evidenced by 593 the wireline logs (Figure 1). Compared to BA1A, BA1D has lower calculated propor-594 tions of $Mg^{2+} - HCO_3^-$ water (only $2.8 \% Mg^{2+} - HCO_3^-$ in the 45 m to 75 m interval and $1.9 \% Mg^{2+} - HCO_3^-$ in the 102 m to 132 m interval; Table S1; Figure 2). This is 595 596 consistent with the lack of evidence of downflow within BA1D. The collection of near 597 end-member $Ca^{2+}-OH^{-}$ fluids from these deeper intervals in BA1D indicates that the packer system is a promising tool for retrieving end-member $Ca^{2+}-OH^-$ waters from 599 deep ophiolite aquifers, even when these aquifers are overlain by aquifers containing Mg^{2+} -600 HCO_3^- waters. 601

5.2 Dissolved gas dynamics

602

Before interpreting our gas chemistry results, the possibility of degassing during sampling must be addressed. Bubbles were observed in the pumped outflow when pumping the hyperalkaline intervals. In addition, interferences with in-line flow meter (Figure S2) readings were observed during pumping of BA1D in 2019, which were rectified by tilting the flow meter at an incline, suggesting that bubbles in the flow meter were causing the unstable readings. These observations suggest that partial gas exsolution may
 have occured upstream of the flow-splitting manifold used for collecting our samples (Fig ure S2). A potential cause of degassing is cavitation associated with the fittings or mea surement mechanisms of the flow meter.

That said, some simple calculations of tubing diameters and flow rates suggest that 612 degassing probably had a minor effect on our data. The black tubing connecting from 613 the pipe string outlet to the flow meter, and then downstream towards the flow-splitting 614 manifold (Figure S2), had a diameter of $\sim 2.5 \,\mathrm{cm}$ and a length of $\sim 10 \,\mathrm{m}$. Given our 615 typical flow rates of $20 \,\mathrm{L}\,\mathrm{min}^{-1}$, the residence time of water in this tubing was 15 s, sug-616 gesting that kinetics of exsolution would have limited degassing over the short time pe-617 riod between fluids exiting the pipe string and arriving at the gas sampling apparatus. 618 Further, noting that packed intervals were generally pumped for an hour or more prior 619 to geochemical sampling, more than 200 tubing-volumes of water were pumped through 620 the tubing prior to sampling (assuming flow of $20 \,\mathrm{L}\cdot\mathrm{min}^{-1}$ for $60 \,\mathrm{min}$). This suggests 621 that, if gas bubbles were present at some locations within the sampling tubing or flow 622 meter, they would have had time to approach equilibrium with gases in the pumped wa-623 ters, resulting in gas partial pressures in the sampled fluids approaching those of the flu-624 ids in the pumped interval. Thus, while we cannot strictly rule out that degassing af-625 fected our measured gas concentrations and isotopic compositions, these calculations sug-626 gest that our data are likely representative and informative. Still, the gas concentrations 627 reported here should be considered minimum values until future work in which gas sam-628 ples are taken closer to the wellhead at BA1A and BA1D corroborates our results. 629

The concentration of H₂ at BA1A and BA1D, which ranged up to $4 \mu \text{mol} \cdot \text{L}^{-1}$ (Table 3), is modest in comparison to other hyperalkaline waters in the ophiolite. Well NSHQ14, which is located 2 km down-gradient within the same catchment as BA1A and BA1D, hosts waters of pH ~ 11.4 with at least two orders of magnitude higher c_{H_2} (A. Paukert, 2014; Nothaft et al., 2020) than the maximum c_{H_2} observed at BA1A and BA1D. These differences could stem from variations in reaction extent and/or lithology between the two wells. In addition, microbial H₂ consumption, notably coupled to SO₄²⁻ reduction (Section 4.3), could decrease c_{H_2} at BA1A and BA1D.

The high δ^{13} C value of C₂H₆ (-2.5 % VPDB; Table 4; Figure 4) in sample BA1D_2019_102-132 indicates an abiotic source of C₂H₆, which has also been proposed for C₂H₆ elsewhere in the ophiolite (Fritz et al., 1992; Nothaft et al., 2020). If C₂H₆ is indeed abiotic at BA1D, then at least some of the CH₄ at BA1A and BA1D is likely also abiotic. However, the detection of 16S rRNA gene sequences affiliated with methanogens of the genus *Methanobacterium* at BA1A and BA1D, albeit in low abundances (< 1% of reads; Section 4.3; Figure S6), suggests a potential additional contribution of microbial CH₄.

Perhaps the most remarkable aspect of our gas data is the ${}^{13}C$ and D enrichment 645 of CH₄, particularly in sample BA1A_2019_108-132 (23.9 % VPDB and 45 % VSMOW; 646 Table 4). This isotopic composition on its own, and especially compared to the relatively 647 less ¹³C- and D-enriched (but still quite enriched) samples from BA1D (Figure 3), is hard 648 to explain without invoking microbial CH_4 oxidation (Section 4.2). This inference is fur-649 ther supported by the lower c_{CH_4} and C_1/C_2 ratio of BA1A_2019_108-132 relative to the 650 BA1D samples (Table 3; Figure S3). CH_4 samples from BA1D may have also undergone 651 microbial oxidation to lesser extents. 652

5.3 Microbial ecology

As proposed in Section 5.1, hydrologic and geochemical data indicate that the 41 m to 75 m aquifer at BA1A may have been hyperalkaline and reduced prior to drilling, similar to the geochemical state of BA1D in 2019 at equivalent depths. If this is so, we infer that, prior to drilling, BA1A and BA1D below ~ 40 m hosted a microbial ecosystem dominated by SO_4^{2-} reduction coupled to oxidation of H₂ and/or organic C such as

the μ mol · L⁻¹ concentrations of small molecular weight compounds such as formate, 659 acetate etc. commonly detected in Samail Ophiolite fluids (Rempfert et al., 2017). On-660 going SO_4^{2-} reduction is consistent with the sulfidic smell observed during drilling of BA1A. 661 The high relative 16S rRNA gene abundance (up to 92% of reads) of sequences related 662 to Thermodesulfovibriona, particularly in BA1D and in OM19_BA1A_108_132, suggest 663 that SO_4^{2-} reduction likely continues to be an important microbial process in much of 664 the subsurface intersected by BA1A and BA1D, particularly in the more reduced zones. 665 Metagenome-assembled genomes affiliated with genus Thermodesulfovibrio within Ther-666 modesulfovibrionia suggest that *Thermodesulfovibrio* is widespread in the Samail Ophi-667 olite and is not capable of CO_2 fixation (Templeton et al., this issue). As such, this *Ther*-668 modesulfovibrio population engages in chemolithoheterotrophy, which is an uncommon 669 metabolic strategy but one observed in several archaeal cultivars (Amenabar et al., 2018). 670 This metabolic strategy may be particularly well-suited to $\sum CO_2$ -depleted, hyperalka-671 line conditions, so long as there is a steady source of organic C. Bioenergetic, metage-672 nomic, and metatranscriptomic evidence of bacterial SO_4^{2-} reduction has also been found 673 at other sites of serpentinization such as the Coast Range Ophiolite (Sabuda et al., 2020) 674 and the Lost City Hydrothermal Vent Field, Mid-Atlantic ridge (Lang et al., 2018). More-675 over, low but detectable rates of microbial SO_4^{2-} reduction have been measured in ground-676 waters sampled from wells in the Samail Ophiolite and the Coast Range Ophiolite (Glombitza 677 et al., in revision). In addition, there was notable blackening and sulfurization of the drill 678 chips recovered during BA1A drilling and analyzed by optical and Raman spectroscopy 679 (E. Ellison and A. Templeton, personal communication, 2020), which could have been caused by reaction with sulfidic fluids. 681

The source of the nearly mmol·L⁻¹ levels SO_4^{2-} at BA1D is unclear. One explanation is leaching of SO_4^{2-} salts previously loaded into these rocks during partial serpentinization during near-spreading ridge hydrothermal alteration and/or during obduction. Another is the oxidation of reduced S in the rocks, which can occur either abiotically or biotically during weathering processes in the presence of O₂ or NO₃⁻ (Luther et al., 2011). The reduced sulfur may source from more S-rich gabbros, which are present in intrusive dikes at the BA1 wells, or from sulfide minerals, which are pervasive in partially altered peridotites in the BA1 wells (Lods et al., 2020; P. Kelemen et al., 2020).

S-oxidizing bacteria including relatives of the genera Sulfuritalea and Cupriavidus, 690 as well as the family Rhodocyclaceae, have accounted for particularly high relative abun-691 dances of 16S rRNA gene reads in groundwaters sampled from wells in the Samail Ophi-692 olite that show evidence of mixing of reduced, hyperalkaline waters with more oxidized 693 $Mg^{2+}-HCO_3^-$ waters and/or gabbro-reacted waters, suggesting that microbial S oxi-694 dation occurs at redox interfaces and mixing zones in the ophiolite (Rempfert et al., 2017). 695 In searching the BA1A and BA1D 16S rRNA gene sequences for the S-oxidizing bacte-696 ria noted by Rempfert et al. (2017), sequences related to Rhodocyclaceae and Cupriavidus 697 were found at up to 2% of reads at BA1A, indicating that microbial S-oxidizing processes 698 may occur at BA1A, although the extent of these processes may be minor. We also note that the DrillFoam used in drilling (Section 2.1) is a biodegradable, sulfate-containing 700 compound, so this may have been an additional source of SO_4^{2-} to the system, although it is not necessary to invoke this biodegradation process as the source of SO_4^{2-} , given the 701 702 presence of the natural S reservoirs discussed above. 703

In addition to SO_4^{2-} reduction coupled to H_2 oxidation, secondary anaerobic, au-704 totrophic processes at BA1A and BA1D may include hydrogenotrophic methanogene-705 sis, evidenced by the presence of 16S rRNA genes affiliated with genus Methanobacterium 706 in < 1% of reads (Figure S6; Section 4.3), and homoacetogenesis, evidenced by the pres-707 ence of 16S rRNA genes affiliated with putative acetogens of the class Acetothermiia ((Takami 708 et al., 2012)) in up to 2% of reads (Figure 5). The apparent dominance of SO_4^{2-} reduc-709 tion over methanogenesis at BA1A and BA1D presents an interesting contrast to the rel-710 ative influences of these processes at the nearby (2 km away) and more H₂-rich ($10^2 \mu$ mol-711

 L^{-1} to $10^3 \mu mol \cdot L^{-1}$; (A. Paukert, 2014; Nothaft et al., 2020)) well NSHQ14, where 712 the abundance and activity of methanogens is comparable to, if not substantially greater 713 than, that of SO_4^{2-} reducers (Miller et al., 2016; Rempfert et al., 2017; Fones et al., 2019; 714 Nothaft et al., 2020; Fones et al., 2020; Kraus et al., 2021). This implies the potential 715 existence of a threshold level of production of reduced compounds, such as H_2 or formate 716 (HCOO⁻), in continental, low-temperature serpentinizing settings, at which methano-717 genesis becomes energetically competitive with SO_4^{2-} reduction. The juxtaposition of 718 the BA1 wells vs. NSHQ14 suggests that this transition may occur at H₂ concentrations in the range of $10 \,\mu\text{mol}\cdot\text{L}^{-1}$ to $10^2 \,\mu\text{mol}\cdot\text{L}^{-1}$ in the Samail Ophiolite. Such a transi-719 720 tion has been described in sedimentary settings, but at orders of magnitude lower c_{H_2} 721 $(\sim 5 \text{ nmol} \cdot \text{L}^{-1}; \text{ (Lovley et al., 1994; Kampbell et al., 1998)}).$ The higher apparent c_{H_2} 722 threshold in serpentinizing settings such as the Samail Ophiolite may be a consequence 723 of the stressors of high pH and low CO_2 availability unique to serpentinizing settings. 724 At hyperalkaline conditions, microbes must expend additional energy to regulate cyto-725 plasmic pH and to maintain a proton motive force across the cell membrane to gener-726 ate ATP (Mitchell, 2011; Mulkidjanian et al., 2008). High pH has been shown to be more 727 important than substrate availability in limiting microbial SO_4^{2-} reduction rates in serpentinization-728 influenced waters from the Samail Ophiolite and the Coast Range Ophiolite (Glombitza 729 et al., 2019). One might expect high pH to be an even greater burden for hydrogenotrophic 730 methanogens due to their reliance on CO_2 for both C fixation and as an oxidant in their 731 energy metabolism. Thus, a higher $c_{\rm H_2}$ threshold for competition between SO₄²⁻ reduc-732 ers and methanogens in serpentinizing settings relative to sedimentary settings may re-733 sult from the high pH and related geochemical challenges. 734

Our detection of high relative abundances of 16S rRNA genes related to heterotrophic 735 bacteria capable of respiration using O_2 or NO_3^- , such as Brachymonas and P. lavamen-736 tivorans, in samples of BA1A taken from $< 70 \,\mathrm{m}$ depth (Figure 5; Section 4.3), taken 737 together with the trends of decreasing $c_{\mathrm{NO}_3^-}$ with increasing depth at BA1A (Section 4.1), 738 suggest that heterotrophic, aerobic and/or denitrifying microbial metabolisms may be 739 active in shallow, subsurface regions. Brachymonas and P. lavamentivorans have been 740 detected in 16S rRNA gene surveys of the Samail Ophiolite prior to Oman Drilling Project 741 activities in low relative abundance (typically < 1% of reads and not exceeding 6%; (Miller 742 et al., 2016; Rempfert et al., 2017; Nothaft et al., 2020; Kraus et al., 2021)), but these 743 taxa were not detected in samples of the drilling fluids used at BA1A (Sections 4.3 and 744 S1: Figure S8). This suggests that these taxa are native to the Samail Ophiolite rather 745 than exogenous drilling contaminants. Natural Brachymonas and P. lavamentivorans 746 populations may have bloomed in response to an influx of oxidized water at depth when 747 previously separated aquifers at different depths were connected by drilling at BA1A (Sec-748 tion 3). Interestingly, *P. lavamentivorans* has been noted for its ability to degrade syn-749 thetic laundry surfactants (Schleheck et al., 2011), suggesting that it could have partic-750 ipated in the biodegradation of DrillFoam (Section 2.1) introduced into the aquifer dur-751 ing drilling. 752

As discussed in Sections 4.2 and 5.2, there is strong isotopic evidence of microbial 753 CH_4 oxidation in fluids accessed by both wells, and especially at BA1A (Figure 3). A 754 search of 16S rRNA gene sequences related to cultured CH_4 oxidizers yielded few matches 755 at BA1A (< 1% of reads) and none at BA1D. Also, all matches are related to Bacte-756 ria thought to exclusively use O_2 to oxidize CH_4 (Figure S6; Section 4.3). It is possible 757 that microbial CH₄ oxidizers are rare at BA1A and BA1D, particularly compared to dom-758 inant taxa such as Thermodesulfovibrionia. CH_4 oxidation may be a minor process that 759 could have been briefly stimulated by O₂ influx during drilling. Alternatively, there may 760 be organisms at BA1A and BA1D whose capacity to oxidize CH_4 under anaerobic con-761 ditions has not yet been documented. Future work could employ shotgun metagenomic 762 sequencing to generate a database of genes to examine for those related to CH_4 oxida-763 tion (e.g., *pmoA* for aerobic methanotrophs, *mcrA* for anaerobic methanotrophs). Al-764 ternatively, microcosm assays or enrichment cultures from BA1A and BA1D that are amended 765

with CH_4 and one of several oxidants (e.g., O_2 , SO_4^{2-}) could be used to detect activity and/or identify microbes with CH_4 oxidation capabilities.

768

5.4 Isotopic composition of $\sum CO_2$

Ophiolitic $Mg^{2+}-HCO_3^-$ waters are in open-system communication with the at-769 mosphere (Neal & Stanger, 1985; Bruni et al., 2002; Cipolli et al., 2004; A. N. Paukert 770 et al., 2012; Leong & Shock, 2020). This is also true of seawater, which sets the δ^{13} C of 771 seawater $\sum CO_2$ at 0 % VPDB to 2 % VPDB, reflecting a series of isotopic equilibria con-772 necting HCO_3^- , the dominant $\sum CO_2$ species in seawater, to the isotopic composition 773 of atmospheric CO₂ (Zeebe & Wolf-Gladrow, 2001). Yet, the δ^{13} C values of $\sum CO_2$ in 774 the $Mg^{2+}-HCO_3^-$ waters in the upper 70 m of BA1A (Table 4; Section 4.2) and in other 775 $Mg^{2+}-HCO_3^-$ waters pumped from wells in the ophiolite (Matter et al., 2006; Nothaft 776 et al., 2020) are $\sim 15 \,\%$ lower than that of marine $\sum CO_2$. This discrepancy raises the 777 question of whether equilibrium with atmospheric CO_2 is the only factor affecting $\sum CO_2$ 778 in ophiolitic $Mg^{2+} - HCO_3^-$ waters. 779

In freshwater systems, respiration of organic matter in soils can be an important 780 factor affecting $\delta^{13}C_{\sum CO_2}$ (Waldron et al., 2007). However, vegetation and soil cover 781 are sparse in the Samail Ophiolite (Figure S1), so it may not be valid to assume that respiration in soils has the capacity to affect $\delta^{13}C_{\sum CO_2}$ to the extent that is observed. How-782 783 ever, organic C within the crystalline bedrock may be oxidized by microbes, thereby pro-784 ducing ¹³C-depleted $\sum CO_2$. Total organic C in peridotites exposed to alteration at the 785 seafloor, a proxy for organic C endogenous to the Samail Ophiolite, is relatively ¹³C-depleted 786 (approximately $-25\pm5\%$ VPDB; (Alt et al., 2013; Alt, Garrido, et al., 2012; Alt, Shanks, 787 et al., 2012; Delacour et al., 2008)). CH₄ in fluid inclusions (Kelley, 1996; Kelley & Früh-788 Green, 1999; Miura et al., 2011; Klein et al., 2019; Grozeva et al., 2020) is another potential source of reduced C. Microbial respiration of these endogenous sources of reduced 790 C could decrease $\delta^{13}C_{\sum CO_2}$ below equilibrium with atmospheric CO₂ if this respira-791 tion occurs at rates comparable to or faster than air-water CO_2 exchange. 792

This hypothesis should be testable by measuring the ¹⁴C content of $\sum CO_2$ in Mg²⁺-793 HCO_3^- waters waters. To our knowledge, there is only one such analysis in the litera-794 ture on the Samail Ophiolite. This is from well WDA17, which is situated in peridodite, has a groundwater pH of 9.10, $c_{\sum CO_2}$ of 2.481 mmol·kg⁻¹, $\delta^{13}C_{\sum CO_2}$ of -12.3 % VPDB, $F^{14}C_{\sum CO_2}$ of 0.205 (corresponding to a ¹⁴C age of 12700 years B.P.), and a ³H/³He recharge 795 796 797 age of 21.5 years (Paukert Vankeuren et al., 2019). It is interesting that the ¹⁴C "age" 798 of the $\sum CO_2$ in this sample is considerably older than its ${}^{3}H/{}^{3}He$ -derived recharge age 799 and that its $\delta^{13}C_{\sum CO_2}$ is well below that which would be expected based on carbon-800 ate equilibrium with atmospheric CO_2 . These characteristics are compatible with the 801 hypothesis of recent microbial oxidation of ancient organic matter stored in the partially-802 serpentinized peridotites. 803

An alternative explanation would be that the ¹⁴C-free C derives from dissolution/reprecipitation of carbonate veins that are ¹⁴C-free. Such veins could have inherited C from marine organic C through thermochemical sulfate reduction (Goldstein & Aizenshtat, 1994), which can produce relatively ¹³C-depleted inorganic C. However, this suggestion is complicated by the fact that calcite, dolomite, and magnesite are typically at or above saturation in $Mg^{2+}-HCO_3^-$ waters in the Samail Ophiolite (Neal & Stanger, 1985; Matter et al., 2006), and magnesite should precipitate (rather than dissolve) from these waters during reaction with peridotite (Bruni et al., 2002; A. N. Paukert et al., 2012).

⁸¹² The ¹³C depletion of $\sum CO_2$ below equilibrium with atmospheric CO₂ in Mg²⁺ – ⁸¹³ HCO₃⁻⁻ waters could alternatively be explained by these waters expressing a muted sig-⁸¹⁴ nal of the kinetic isotope effects associated with hydroxylation of aqueous CO₂, which ⁸¹⁵ have been invoked to explain the stark ¹³C depletion in Ca-rich carbonate travertine deposits found at hyperalkaline springs in ophiolites (Clark & Fontes, 1990; Clark et al., 1992; P. B. Kelemen et al., 2011; Falk et al., 2016). However, this seems unlikely because a rapid CO₂ uptake process would be necessary to achieve these kinetic isotopic effects. Further, in contrast to the travertine deposits, there is no clear mechanism to preserve such a signature in a $Mg^{2+}-HCO_{3}^{-}$ water (as in a precipitation process), so CO₂ (aq) and HCO_{3}^{-} would be expected to quickly re-equilibrate.

Although we cannot resolve the compositional and isotopic dynamics of inorganic C in $Mg^{2+}-HCO_3^-$ waters given the available data, this discussion has highlighted important aspects of the C cycle of low-temperature serpentinizing systems that are ripe for future investigation. Further analyses of the stable and radio isotopic compositions of inorganic C in ophiolitic $Mg^{2+}-HCO_3^-$ waters, especially if coupled to ${}^{3}H/{}^{3}He$ -derived recharge ages, could advance understanding of the timescales and sources of CO_2 uptake in near-surface serpentinizing aquifers.

6 Conclusions

In this study, vertically- and horizontally-resolved sample acquisition via the de-830 ployment of a packer system in two 400 m-deep wells enabled a holistic geochemical, hy-831 drologic, and biological investigation into an aquifer experiencing ongoing low-temperature 832 serpentinization. In addition, a temporal component was assessed by monitoring sub-833 surface biogeochemical states from the time of well completion to three years afterwards. 834 Through the isolation and pumping of discrete intervals as deep as 108 m to 132 m be-835 low ground level, we interrogated how microbial communities of minimally-disturbed, 836 hyperalkaline, and reduced groundwaters differed from those of pervasively mixed and 837 oxidized groundwaters. Aqueous chemical analyses and 16S rRNA gene sequencing of 838 deep, $Ca^{2+}-OH^-$ groundwaters revealed the presence of a microbial ecosystem dom-839 inated by organisms potentially supported by H_2 oxidation coupled to SO_4^{2-} reduction, 840 with small organic acids generated through sestpentinization possibly serving as an or-841 ganic C source. Based on these findings, we propose that future investigations of the bore-842 hole lithology seek evidence for late-stage sulfurization induced by microbial activity. In 843 oxidized $Mg^{2+}-HCO_3^-$ groundwaters, heterotrophic bacterial aerobes and/or denitri-844 fiers were dominant. This may be a common state for the shallow aquifers, or it may have 845 been stimulated by drilling-induced groundwater mixing. Isotopic data point to intrigu-846 ing future avenues of C cycle investigations in this system. Stark ¹³C and D enrichments in CH₄ and ¹³C depletions in $\sum CO_2$ of Mg²⁺-HCO₃⁻ groundwaters below the expec-847 848 tation of equilibrium with atmospheric CO_2 suggest the importance of cryptic microbial 849 oxidation of stored C reservoirs. Overall, the methodology and results of this study mark 850 an important step towards an integrated hydrologic and biogeochemical understanding 851 of the serpentinite-hosted biosphere. As such, this study presents a framework for ex-852 ploring where subsurface energy availability is greatest and how that maps onto micro-853 bial abundance and activity, information that is needed for improving strategies for life 854 detection in fractured rock aquifers on and beyond Earth. 855

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⁸⁸¹ Data (in Excel format) and source code (in R Markdown format) used to produce the figures, data tables and analyses for this paper (as well as additional data on analytical uncertainties and trace element concentrations) are available online at https:// github.com/danote/Oman-packers-2018-2019. Additional DNA sequence data processing codes are available at https://github.com/danote/Samail_16S_compilation.

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Supporting Information for Aqueous geochemical and microbial variation across discrete depth intervals in a peridotite aquifer assessed using a packer system in the Samail Ophiolite, Oman

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³⁰ S1. Supplementary 16S rRNA gene sequencing

Two 1 L autoclaved glass bottles were filled with drill foam/fluid that had surfaced after subsurface circulation during drilling of BA1A in 2017. The drilling foam and fluid was filtered through $0.22 \,\mu m$ polycarbonate filters at Colorado School of Mines. The drill foam/fluid samples totaled 1.5 L in volume and were split into two replicates, resulting in 0.75 L of foam/fluid filtered for each replicate. Nucleic acids concentrated onto the filters were extracted, amplified, and sequenced as described by Kraus et al. (2018). The drill foam/fluid samples (Figure S8) show very little taxonomic overlap with the fluids samples with packers (Main Text Figure 5).

In addition, a cell size fractionation experiment was performed for biomass filtering of BA1A in 2018. The sequential in-line filter housings described in the main text correspond to the three white cylinders near the bottom of Figure S2. Main Text Figure 5 shows the results of sequencing $0.22 \,\mu$ m pore-diameter filters only. Results of sequencing filters of all pore diameters are reported in (Figure S7).

⁴² S2. Tables

Table 51: Mixing extents based on Si, after Leong et al. (2020).												
Sample ID	$\sum Si / \left[\mu mol \cdot L^{-1} \right]$	Mixing extent / [% of $Mg^{2+} - HCO_3^-$ water]										
BA1A_2018_55-66	1.97×10^2	65										
BA1A_2018_100-400	4.49×10^1	15										
BA1A_2019_0-30	3.33×10^2	110 ^a										
BA1A_2019_41-65	1.56×10^2	51										
BA1A_2019_108-132	2.13×10^1	7.0										
BA1D_2019_45-75	8.51	2.8										
BA1D_2019_102-132	5.88	1.9										

Table S1: Mixing extents based on Si, after Leong et al. (2020).

^aBA1A_2019_0-30 has a calculated mixing extent > 100 % we performed these calculations using the same $Mg^{2+} - HCO_3^-$ end member as Leong et al. (2020), which had a $c_{\sum Si}$ of 303 μ mol·kg. This sample should be considered representative of a typical $Mg^{2+} - HCO_3^-$ water.

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43 S3. Figures



Figure S1: Packer installation at BA1D, January, 2019. The orange tripod, installed at BA1D in the photo, was used to suspend the packer assembly down hole. The wellhead of BA1A can be seen 15 m to the right of BA1D in the photo. The third rotary well at the BA1 site, BA1C, which collapsed shortly after drilling, is pictured in the background. The cored borehole, BA1B, is 120 m to the northwest, to the left of the frame.



Figure S2: Fluid pumping and sampling at BA1A, February, 2019. Labeled arrows indicate the top of the pipe string, from which the pumped water flowed, the flow meter used for hydrologic pump tests, and the flow-splitting manifold used for fluid and biomass sampling.



Figure S3: Plot of ratio of methane (C₁) to the sum of ethane (C₂) and propane (C₃) vs. $\delta^{13}C_{CH_4}$. Only analyses for which C₂ was above limit of quantitation are plotted. If C₃ was below limit of quantitation, its contribution to C₁/(C₂ + C₃) was assumed to be negligible, and therefore C₁/C₂ is plotted. Shaded fields of typical gas origin after Milkov and Etiope (2018). Contextual data from Samail Ophiolite from Nothaft et al., 2020; Etiope et al., 2015; Vacquand et al., 2018. Abbreviations: PM, primary microbial; SM, secondary microbial; T, thermogenic; A, abiotic.



Figure S4: Plot of Oman groundwater stable isotopic compositions. Samples from 2012 were reported in Paukert Vankeuren et al. (2019). Samples from 2014 reported in Miller et al. (2016). Samples from 2018 (apart from BA1A) reported in Nothaft et al. (2020) *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 S5: SO_4^{2-} concentrations in Samail Ophiolite wells. Data from Miller et al., 2016; Rempfert et al., 2017; Kraus et al., 2018; Nothaft et al., 2020.



Figure S6: 16S rRNA gene read relative abundances of DNA extracted from filter-concentrated groundwaters from BA1A and BA1D affiliated with CH_4 -cycling taxa. 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, but when that taxon was detected in 16S gene reads of other Oman samples obtained during the same sampling year, are labeled "n.r." Cases when no reads were detected in any Oman sample within the data set of a given year are blank."

							I	De	ере	əst	tax	on	on	nic	as	sig	nm	en	t					
			Other taxa -	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Hydrogenophilaceae; g_Thiobacillus; s_thioparus -	k_Bacteria; p_Cyanobacteria; c_Vampirivibrionia; o_Caenarcaniphilales; f_NA; g_NA; s_NA -	k_Bacteria; p_Patescibacteria; c_Parcubacteria; o_Candidatus_Jorgensenbacteria; f_NA; g_NA; s_NA -	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Rhodocyclaceae; g_Methyloversatilis; s_NA -	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Sphingomonadales; f_Sphingomonadaceae; g_Sphingopyxis; s_NA -	k_Bacteria; p_Bacteroidota; c_Ignavibacteria; o_Ignavibacteriales; f_SR-FBR-L83; g_NA; s_NA -	k_Bacteria; p_Nitrospirota; c_Nitrospiria; o_Nitrospirales; f_Nitrospiraceae; g_Nitrospira; s_inopinata -	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Pseudomonadaceae; g_Pseudomonas; s_NA-	k_Archaea; p_Nanoarchaeota; c_Nanoarchaeia; o_Woesearchaeales; f_GW2011_GWC1_47_15; g_NA; s_NA-	k_Bacteria; p_Chloroflexi; c_P2-11E; o_NA; f_NA; g_NA; s_NA -	k_Baderia; p_NA; c_NA; c_NA; c_NA; g_NA; s_NA-	k_Bacteria; p_Patescibacteria; c_Parcubacteria; o_NA; f_NA; g_NA; s_NA -	k_Archaea; p_Crenarchaeota; c_Nitrososphaeria; o_Nitrosopumilales; f_Nitrosopumilaceae; g_Candidatus_Nitrosotenuis; s_NA-	$_Archaea; p_Crenarchaedta; c_Nitrososphaeria; o_Nitrososphaerales; f_Nitrososphaeraceae; g_Candidatus_Nitrososphaera; s_NA-Crenarchaedta; b_Na+Crenarchaedta; b_Na+C$	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Comamonadaceae; g_Azohydromonas; s_NA-	k_Bacteria; p_Verrucomicrobiota; c_Verrucomicrobiae; o_Opitutales; f_Opitutaceae; g_Lacunisphaera; s_NA-	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_NA; f_NA; g_NA; s_NA -	k_Bacteria; p_Deinococcota; c_Deinococci; o_Thermales; f_Thermaceae; g_Meiothermus; s_NA -	k_Bacteria; p_Nitrospirota; c_Thermodesulfovibrionia; o_NA; f_NA; g_NA; s_NA -	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Comamonadaceae; g_Brachymonas; s_NA -	
at R	т	0.10	11	n.r.	n.r.	n.r.	-	7	7	-	-	n.r.	n.r.	7	7	n.r.	n.r.	-	n.r.	-	ω	-	80	
ead relat oundance	ilter pore depth o	0.22	21	7	7	n.r.	-	-	n.r.	2	-	n.r.	n.r.	7	7	-	7	ω	7	4	22	33	6	100-400
ive ∍ / [%] ₀	e diamete of sampli	0.45	26	7	n.r.	n.r.	2	-	n.r.	2	-	7	n.r.	7	n.r.	7	7	ъ	n.r.	-1	26	25	11	
20	er / [µm] ing inte	0.10	17	2	2	ω	7	-	_	n.r.	7	ω	2	2	ъ	ω	2	7	4	4	Сл	17	26	
40] group rval / [n	0.22	13	-	N		7	-	-	n.r.	7	2	2	2	2	2	ω	7	6	4	Сл	20	32	55-6
i 80	ed by م]	0.45	11	-	7	7	-	-	2	n.r.	-	-	-	-	7		ω	7		2	7	21	44	6

concentrated using inline filters of sequentially decreasing pore diameters from well BA1A in 2018. Read relative abundances are reported as percentages rounded to when no reads were detected in any Oman sample within the data set of a given year are blank." the ones place. Figure S7: were detected in a sample, but when that taxon was detected in 16S gene reads of other Oman samples obtained during the same sampling year, are labeled "n.r." Cases 16S rRNA gene read relative abundances of 20 most abundant taxonomic assignments in DNA extracted from groundwaters from which biomass was 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

8

	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria, o_Pseudomonadales; f_Pseudomonadaceae; g_Pseudomonas; s_NA	39	41	Ļ	ę	
	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_Caulobacteraceae; g_Brevundimonas; s_balnearis -	16	20	18	20	
	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_Caulobacteraceae; g_Phenylobacterium; s_koreense -	6	9	33	19	
	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Comamonadaceae; g_Schlegelella; s_aquatica	9	m	24	17	
	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Sphingomonadales; f_Sphingomonadaceae; g_Sphingopyxis; s_NA	e	S	ĸ	14	
1	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_Caulobacteraceae; g_Phenylobacterium; s_NA	2	-	11	80	
ຸມອເ	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Rhodocyclaceae; g_Methyloversatilis; s_NA -	4	9	0	e	
uuɓ	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_Caulobacteraceae; g_Brevundimonas; s_NA	2	4	-	с	
jissi	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Moraxellaceae; g_Acinetobacter; s_NA -	5	Э	Ý	n.r.	
s oi Î	Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Rhizobiaceae; g_Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium; s_NA -	-		Ŷ	4	
wou	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Acetobacterales; f_Acetobacteraceae; g_Roseomonas; s_eburnea -	-	n.r.	-	4	
JOX	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Burkholderiales; f_Burkholderiaceae; g_Cupriavidus; s_gilardii -	-	-	-	2	
et te	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Sphingomonadales; f_Sphingomonadaceae; g_Porphyrobacter; s_NA -	2	з	n.r.	n.r.	
sədə	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Sphingomonadales; f_Sphingomonadaceae; g_Blastomonas; s_NA	-	0	n.r.	n.r.	
əəd	kBacteria; p_Proteobacteria; cGammaproteobacteria; oBurkholderiales; fComamonadaceae; gSchlegelella; s_NA -	Ŷ	7	2	-	
I	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Pseudomonadaceae; g_Pseudomonas; s_alcaligenes -	2	n.r.	n.r.	n.r.	
	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Xanthomonadales; f_Xanthomonadaceae; g_Vulcaniibacterium; s_thermophilum -	-	7	v	7	
	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_NA; g_NA; s_NA -	n.r.	n.r.	+	-	
	k_Bacteria; p_Actinobacteriota; c_Actinobacteria; o_Micrococcales; f_Promicromonosporaceae; g_Isoptericola; s_NA	-	n.r.	n.r.	n.r.	
	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Beijerinckiaceae; g_Chelatococcus; s_caeni -	n.r.	n.r.	4	n.r.	
	- Other taxa	4	3	2	2	
		Re	splicate grouped	d by nucleic acid		
		Read I abund	relative ance / [%]	8		
			0.	10 20 30	40	

drill foam / fluid effluent from BA1A, acquired during drilling in 2017. Read relative abundances are reported as percentages rounded to the ones place. Cases when a Figure S8: 165 rRNA gene read relative abundances in of 20 most abundant taxonomic assignments in DNA and cDNA (from RNA) (Kraus et al., 2018) extracted from 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, but when that taxon was detected in 16S gene reads of other Oman samples obtained during the same sampling year, are labeled "n.r." Cases when no reads were detected in any Oman sample within the data set of a given year are blank.

RNA

DNA

	[2018	BA1A				2019 BA1	A	2019	BA1D
_ at	fRhodocyclaceae; gMethyloversatilis; sNA -	1	1	2	<1	<1	1					
pe j	f_Rhodocyclaceae; g_Dechlorosoma; s_NA -	<1	1	2	n.r.	n.r.	n.r.	<1	n.r.	n.r.	n.r.	n.r.
lee lee	f_Rhodocyclaceae; g_Azospira; s_oryzae -	n.r.	<1	<1	<1	<1	<1	<1	<1	n.r.	n.r.	n.r.
ass d c	fRhodocyclaceae; gNA; sNA -	n.r.	n.r.	n.r.	n.r.	<1	n.r.	<1	n.r.	n.r.	n.r.	n.r.
an	fRhodocyclaceae; gDenitratisoma; sNA -	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	<1	n.r.	<1	n.r.	n.r.
uly of	fBurkholderiaceae; gCupriavidus; sgilardii -	<1	n.r.	n.r.	n.r.	n.r.	n.r.					
an	fBurkholderiaceae; gCupriavidus; sNA -	n.r.	n.r.	<1	n.r.	n.r.	n.r.					
τaχ τ	f_Rhodocyclaceae; g_Dechloromonas; s_agitata -	n.r.	n.r.	<1	n.r.	n.r.	n.r.					
'	1	100-400	100-400	100-400	55-66	55-66	55-66	0-30	108–132	41–65	102–132	45–75
		0.10	0.22	0.45	0.10	0.22	0.45	0.22	0.22	0.22	0.22	0.22
			Dept	h of sai	nolina	interva	l / [m] ar	nd filter	pore di	ameter	/ [um]	

grouped by year of sampling and well name

Figure S9: 16S rRNA gene read relative abundances of DNA extracted from filter-concentrated groundwaters from BA1A and BA1D affiliated with S-oxidizing taxa noted by Rempfert et al. (2017) (presented at family level and deeper). 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, but when that taxon was detected in 16S gene reads of other Oman samples obtained during the same sampling year, are labeled "n.r." Cases when no reads were detected in any Oman sample within the data set of a given year are blank."

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