

Contents lists available at ScienceDirect

Earth and Planetary Science Letters



www.elsevier.com/locate/epsl

Potential energy sources for the deep continental biosphere in isolated anoxic brines



William S. Dowd^a, Christopher J. Schuler^b, Cara M. Santelli^b, Brandy M. Toner^{b,c}, Cody S. Sheik^d, Kelden Pehr^a, Jill M. McDermott^{a,*}

^a Department of Earth and Environmental Sciences, Lehigh University, Bethlehem, PA, United States

^b Department of Earth and Environmental Sciences, University of Minnesota Twin Cities, Minneapolis, MN, United States

^c Department of Soil, Water, and Climate, University of Minnesota Twin Cities, St. Paul, MN, United States

^d Department of Biology and Large Lakes Observatory, University of Minnesota Duluth, Duluth, MN, United States

ARTICLE INFO

Article history: Received 24 February 2022 Received in revised form 26 May 2022 Accepted 5 July 2022 Available online xxxx Editor: B. Wing

Keywords: deep biosphere anoxic brine Gibbs free energy alkanes clumped methane dimethylamine

ABSTRACT

In isolated fracture networks in the Precambrian Shield, long-term water and rock interactions produce saline anoxic fluids that host extant microbial communities deep within the continental subsurface. Light and oxygen (O_2) are absent in these environments. Thus, chemotrophic organisms inhabiting these systems rely on anaerobic reactions for energy. Viable electron donors include short-chain alkanes, such as methane (CH₄) and C_{2+} alkanes, while alternative electron acceptors include sulfate (SO₄²⁻), nitrate (NO_3^{-}) , and ferric iron (Fe^{3+}) . Here, we constrain the potential sources of energy for microorganisms in Neoarchean bedrock on the 27th level west drift of the Soudan Underground Mine State Park, MN, USA (713.5 meters below the surface). The Gibbs Free Energy (ΔG) of 11 reactions are modeled and expressed as available chemical potential energy per mass fluid (J/kgfluid). Metabolic reactions involving CH_4 oxidation by SO_4^{2-} would yield the highest potential energy of reactions modeled in this study (-111 J/kg_{fluid}). The free energy for methanogenesis via the breakdown of dimethylamine (DMA; \sum (CH₃)₂NH_(aq)) is exergonic but with near-zero available energy per mass fluid, suggesting that DMA may be cycled quickly to produce biological CH₄ at Soudan. We examine all the possible pathways by which CH₄ and other short-chain alkanes may be formed. Conventional $\delta^{13}C_{CH4}$ values and C1/C2+ abundance ratios support a mixed biological and non-biological origin of CH4. Doubly substituted 'clumped' CH₄ isotope ¹³CH₃D values are consistent with formation temperatures of 84-89 °C that exceed current environmental conditions of 11.5-12.1 °C. These estimated formation temperatures are too low for CH₄ to be formed solely through thermogenic degradation of organic matter. Further, low or undetectable H₂ rules out active abiogenesis of CH₄ from CO₂ reduction. It is more likely that the bulk CH₄ pool reflects a mixture of microbial CH₄ with Δ^{13} CH₃D values equilibrated at 11.5-12.1 °C and thermogenic CH₄ formed at temperatures > 100 °C. Understanding the origin and cycling of these electron donors contributes to a fundamental understanding of how microbial activity may promote, maintain, or suppress the habitability of these isolated systems over long timescales.

© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Life in the continental subsurface makes up an estimated \sim 2-19% of Earth's total global biomass (McMahon and Parnell, 2014). Phylogenetically diverse microbial communities can inhabit deep continental reservoirs hosted in bedrock fracture networks that extend to depths of >3 km below Earth's surface (Onstott et al., 2003; Sheik et al., 2021). Due to physical isolation imposed by a hydrologic disconnect from surficial waters, nutrients and carbon in these environments can be scarce (Holland et al., 2013). Nonetheless, microorganisms that adapt to these conditions can reproduce and maintain key cellular functions (Lever et al., 2015).

Successful microbial energy production in the isolated subsurface hinges on utilizing available geochemical electron donors and acceptors. These oxidation-reduction (redox) couples are mediated by water and rock interactions and microbial metabolic activity. Long-term chemical reactions between water and rock in Precambrian bedrock yield high density, anoxic brines rich in calcium (Ca^{2+}) and chloride (Cl^-) (Frape et al., 1984). Due to a lack of dissolved free molecular oxygen (O_2), redox reactions in these fluids

https://doi.org/10.1016/j.epsl.2022.117720

^{*} Corresponding author. E-mail address: jill.mcdermott@lehigh.edu (J.M. McDermott).

⁰⁰¹²⁻⁸²¹X/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

require alternative oxidants (e.g., SO_4^{2-} , NO_3^{-} , Fe^{3+}) to function as electron acceptors. In contrast, electron donors are readily available in Precambrian Shield brines in the form of C₁-C₄ alkanes (Sherwood Lollar et al., 1993a), more complex organics such as acetate (Sherwood Lollar et al., 2021), and H₂ (Li et al., 2016). Significant quantities of alkanes such as methane (CH₄), ethane (C₂H₆), propane (C₃H₈), *i*-butane (*i*-C₄H₁₀), and *n*-butane (*n*-C₄H₁₀) are emitted from boreholes drilled into deep mines in the Canadian Shield (Sherwood Lollar et al., 2002), Fennoscandian Shield (Nurmi et al., 1988), and South African Shield (Ward et al., 2004). These sites host ancient brine-filled reservoirs with mean residence times spanning tens of thousands to billions of years (Holland et al., 2013).

The formation of C_1-C_4 alkanes in any natural system can be linked to three processes: the biological reduction of carbon to CH₄ (i.e., methanogenesis), the thermal degradation of organic matter (i.e., thermogenesis), and the abiotic polymerization of C-H bonds through catalytic reactions such as Fischer-Tropsch-type (FTT) synthesis (i.e., abiogenesis) (Sherwood Lollar et al., 2002; McCollom and Seewald, 2007). As such, distinguishing the production pathway(s) of C₁-C₄ alkanes in low-temperature systems is complex because measured abundances reflect the net effect of carbon cycling and isotopic exchange through microbial manipulation and mixing of thermogenic and/or abiotic sources (Reeves and Fiebig, 2020). Each of these mechanisms has been invoked as the origin of C₁-C₄ alkanes in Precambrian Shield sites (Sherwood Lollar et al., 2002; Ward et al., 2004).

The Soudan Underground Mine State Park, MN, USA, contains a high salinity, anoxic reservoir hosted in Neoarchean (>2.5 Ga) bedrock (Hudak et al., 2016) that provides an opportunity to investigate the controls on the habitability of the deep continental crust. Three legacy boreholes on the 27th level (713.5 meters below the surface) tap the reservoir and act as conduits for gas and fluid. First, we identify possible energy sources that may support microbial communities hosted in Soudan's anoxic brines. Favorable reactions for extant microbial communities are quantified using a thermodynamic approach applied to our measured compositions. Second, we discuss the formation mechanisms that produce C1-C4 alkanes in the system. Our study on biogeochemical cycling at the Soudan Mine will aid in resolving habitability constraints for Earth's continental subsurface. It will also provide a baseline for processes occurring in analogous bedrock fracture systems on other planets, such as the subsurface of Mars.

2. Geologic setting

The Soudan Underground Mine State Park is located in the southwest region of the Superior Craton in northern Minnesota's Vermilion District. The Vermilion Greenstone Belt, on the western end of the Wawa-Abitibi Terrane, hosts the Ely Greenstone Formation, Lake Vermilion Formation, and the Soudan Iron Formation (Hudak et al., 2016), the latter extending through the 27th level west drift of the mine 713.5 meters below the surface. The bedrock is composed of Neoarchean (>2.5 Ga) hematite/jasper banded iron formation (BIF) interlayered with hydrothermally altered basaltic flows (Thompson, 2015) consisting of chlorite schist and massive hematite ore. Our study was conducted ~650-800 m down the 27th level west drift at three slightly angled downward legacy boreholes. The three borehole orifices are spaced approximately 50 m apart and extend to depths of 102-144 m below the mine floor (Fig. 1). Each borehole taps a saline fluid-filled fracture network and emits fluid and gas at the surface of the drift. Diamond drilled holes (DDH) DDH951 and DDH932 intersect large bodies of chlorite schist with minimal Fe-bearing deposits. The most western borehole in this study, DDH944, consists of shallow potions of BIF with hematite ore at deeper depths (Schuler et al., 2022).

3. Materials and methods

3.1. Sample collection

Gas samples were collected by submerging an inverted beaker equipped with a rubber septum at the mouth of each borehole, displacing the beaker volume with upwelling brine and allowing gas to collect. Gases were transferred into previously evacuated 30 mL borosilicate glass vials using plastic syringes and over-pressured to approximately triple the volume of the vessel. Borosilicate vials prepared for alkanes (CH₄, C₂H₆, C₃H₈, *i*-C₄H₁₀, *n*-C₄H₁₀) and inorganic gases (H₂, N₂, Ar, O₂, He) were pretreated with HgCl₂, evacuated to at least 60 mTorr vacuum, and sealed with NaOH-boiled blue butyl rubber stoppers. Vials for $CO_{2(g)}$ were prepared similarly but sealed with black butyl rubber stoppers.

Aqueous samples were collected with a pre-cleaned, autoclaved stainless steel and silicone packer system (Fig. 1) deployed in the top 1-2 m of each borehole. Each packer was inflated to seal off the borehole and minimize atmospheric contamination. Temperature, conductivity (mS), and pH were determined with an Oakton[®] handheld probe calibrated with NIST-traceable conductivity and pH standards. Aliquots for dissolved inorganic carbon $(\sum CO_{2(aq)} = CO_{2(aq)} + HCO_{3^{-}(aq)} + CO_{3^{2^{-}}(aq)})$ were stored in evacuated borosilicate glass vials and sealed with black butyl rubber stoppers. The $\sum CO_{2(aq)}$ samples were then acidified with H₃PO₄ to a pH <2. Aliquots for water isotopes $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ were collected without headspace and stored in HCl-washed highdensity polyethylene (HDPE) NalgeneTM bottles. Total ammonium $(\sum NH_4^+{}_{(aq)} = NH_4^+{}_{(aq)} + NH_{3(aq)})$ samples were filtered with $0.22 \ \mu\text{m}$ polyethersulfone filters and stored frozen in 60 mL amber glass vials pretreated with ZnCl₂. Samples for monomethylamine (MMA; $\sum CH_3NH_{2(aq)} = CH_3NH_{2(aq)} + CH_3NH_3^+(aq)$), dimethylamine (DMA; $\sum(CH_3)_2NH_{(aq)} = (CH_3)_2NH_{(aq)} + (CH_3)_2NH_{2^+(aq)}$), and trimethylamine (TMA; $\sum(CH_3)_3N_{(aq)} = (CH_3)_3N_{(aq)} +$ $(CH_3)_3NH^+_{(aq)})$ were acidified with 10N HCl to a pH of 3, mixed with 120 mg of NH₄Cl, and stored frozen in 2 L fired glass bottles until analysis.

Aqueous samples for solvent extractable organic analysis were collected with a peristaltic pump using solvent cleaned Clearflo 70 and copper tubing which was inserted into the top 1-2 m of each borehole and filtered with a Sterivex 0.22 µm filter. Aliquots for solvent extractable organic analysis were stored frozen in fired 1 L glass bottles.

Water samples for major anion (Cl, Br, SO₄) and cation (Ba, Ca, Fe, K, Li, Mg, Mn, Na, Si, Sr) analyses were collected in sterile syringes and filtered through 0.2 μ m PES membranes into 50 mL plastic sample tubes. Cation samples were preserved with 10 μ L 12 M hydrochloric acid per mL of solution. Water samples were stored on ice after collection, then transferred to 5 °C storage until analysis.

3.2. Analytical methods

A Hewlett Packer 5890 Series II Gas Chromatograph equipped with a flame ionization detector (GC-FID) was used to quantify CH₄, C₂H₆, C₃H₈, *i*-C₄H₁₀, and *n*-C₄H₁₀ with a Supelco 80/100 Porapak Q 6 ft x 1/8 in x 2.1 mm stainless steel column. A He carrier gas was set to a pressure of 20 psi while FID H₂ and zero air pressures were set to 29 psi and 37 psi, respectively. The oven was set to an initial temperature of 30 °C with a 2-minute hold time, followed by heating with a temperature ramp of 12 °C per minute to hold at a final temperature of 220 °C for 2 minutes. Inorganic carbon abundances, including CO_{2(g)} and \sum CO_{2(aq)} were quantified on a Hewlett Packer 5890 Series II Gas Chromatograph equipped with a thermal conductivity detector (GC-TCD) with a Supelco 80/100 Porapak Q 6 ft x 1/8 in x 2.1 mm stainless steel column and a



Fig. 1. Map view of Soudan Mine Underground State Park, MN. (a) Image of the 27th level west drift where fluid and gas were sampled. (b) Arranged west to east are DDH944, DDH951, and DDH932, approximately 50 m apart. Each downward-drilled borehole is angled slightly eastward and extends to depths up to 144 m below the mine floor. (c) The mouth of legacy boreholes where artesian fluid and gas flow up to the surface of the drift. (d) Stainless steel packer systems are inserted into each borehole to access upwelling anoxic waters and minimize atmospheric contamination.

He carrier gas set at 20 psi. The oven was held at an isothermal temperature of 30 °C. Inorganic gases, H₂, N₂, Ar, O₂, and He were quantified with an Agilent 7890B GC-TCD. An HP-Molesieve 30 m x 0.320 mm x 25 µm column was used with a He carrier gas at 2.5 mL/min to quantify O₂, N₂, and Ar at an isothermal temperature of 40 °C for 10 minutes. N₂ carrier gas was used at 1.5 mL/min to quantify H₂ and He at an isothermal temperature of 40 °C for 10 minutes with the same column. All gas samples were run in triplicate with uncertainties (2 σ) of ±5% for CH₄, C₂H₆, C₃H₈, n-C₄H₁₀, CO_{2(g)}, and N₂, and ±10% for *i*-C₄H₁₀, H₂, O₂, He, and Ar.

Concentrations of $\sum NH_4^+(_{aq})$ were determined by flow injection analysis (see **SI** for additional details). Samples were run in triplicate with uncertainties (2σ) of $\pm 5\%$. MMA, DMA, TMA were quantified by solid-phase microextraction (SPME) via headspace extraction (see **SI** for additional details). Samples were run in triplicate with uncertainties (1σ) of $\pm 10\%$.

Stable isotope ratios are expressed in δ notation in per mil units (‰); e.g., for carbon:

$$\delta^{13}C = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000(\%)$$
(1)

where R_{sample} and $R_{standard}$ are the isotope ratios $({}^{13}C/{}^{12}C)$ of both the sample and standard. Stable isotope values for $\delta^{13}C$ of C_1 - C_4 alkanes, $\sum CO_{2(aq)}$, and $CO_{2(g)}$ were determined via isotope ratiogas chromatography mass spectrometry at the Woods Hole Isotope lab, MA, USA and standardized to the Vienna Pee Dee Belemnite scale (VPDB) with uncertainties (2σ) of $\pm 0.8\%$ for $\delta^{13}C_{CO2(g)}$, $\pm 0.8\%$ for $\delta^{13}C_{CO2(aq)}$, $\pm 0.5\%$ for $\delta^{13}C_{CH4}$, $\pm 0.8\%$ for $\delta^{13}C_{C2H6}$, $\pm 0.6\%$ for $\delta^{13}C_{C3H8}$, and $\pm 0.8\%$ for $\delta^{13}C_{nC4H10}$. Stable isotope values for δ^{2} H of CH₄ and C₂H₆ were determined via isotope ratiogas chromatography mass spectrometry at the Woods Hole Isotope lab, MA, USA following the methods described in (Reeves et al., 2012) with uncertainties (2σ) of $\pm 4.0\%$ for δ^{2} H_{CH4} and $\pm 3.0\%$ for δ^{2} H_{C2H6}. After azeotropic distillation, values of δ^{18} O_{H20} and δ^{2} H_{H20} were measured with a Los Gatos Research-OA-ICOS Laser System at the Environmental Isotope Lab Waterloo, Ontario. Values of δ^{18} O_{H20} and δ^{2} H_{H20} for δ^{18} O and $\pm 0.8\%$ for δ^{2} H.

For samples collected in November 2019, doubly substituted 'clumped' CH₄, Δ^{13} CH₃D (‰) values were determined via tunable infrared laser direct absorption spectroscopy using previously described methods in Ono et al. (2014) and Wang et al. (2015) at the Massachusetts Institute of Technology. The value of Δ^{13} CH₃D is the measure of the excess abundance of ¹³CH₃D over stochastic distributions and was calculated based on:

$$\Delta^{13} \text{CH}_3 \text{D} = \ln\left(\frac{{}^{13}\text{CH}_3 \text{D}}{{}^{12}\text{CH}_3 \text{D}} \times \frac{{}^{12}\text{CH}_4}{{}^{13}\text{CH}_4}\right) \times 1000(\%)$$
(2)

(Ono et al., 2014). The precision for Δ^{13} CH₃D was 0.1‰ to 0.3‰ (95% confidence interval).

Borehole #	DDH932	DDH932 (111519)	DDH951	DDH951 (111519)	DDH944
Date	(02.20.13)	(11.13.13)	(02.20.13)	(11.15.15)	(02.20.13)
Temperature (°C)	11.5	11.5	12.1	12.1	11.9
pH	6.1	6.0	6.7	6.1	6.9
Conductivity (mS)	174	159	130	121	159
Water flow rate (mL/s)	-	-	3.3	3.4	1.1
Gas flow rate (mL/s)	-	-	0.36	-	0.18
Cl (mmol/kg)	2130	2150	1460	1470	1910
Br (mmol/kg)	5.16	4.79	3.83	3.37	4.37
SO ₄ (mmol/kg)	1.27	1.09	1.16	1.00	1.17
Ba (µmol/kg)	63.6	209	48.3	199	54.7
Ca (mmol/kg)	560	594	409	422	543
Fe (mmol/kg)	1.82	2.07	1.01	1.24	1.54
K (mmol/kg)	6.47	9.73	4.91	7.25	6.29
Li (µmol/kg)	344	385	251	260	334
Mg (mmol/kg)	95.4	118	76.2	88.5	94.5
Mn (µmol/kg)	360	332	286	253	337
Na (mmol/kg)	525	625	388	421	512
Si (µmol/kg)	155	3700	100	5530	83.7
Sr (mmol/kg)	5.20	8.51	4.01	5.42	5.12
NO ₃ (µmol/kg) ^a	14.3	-	28.6	-	14.3
$\sum CO_{2(aq)} (mmol/kg)$	-	0.314	-	0.307	-
$\overline{\Sigma}$ NH _{4(ag)} (µmol/kg)	35.0	49.6	32.4	20.7	39.5
\sum (CH ₃) ₂ NH (nmol/kg) ^b	-	-	-	41.1	-
$\delta^{13}C_{\sum CO2(aq)}$ (‰)	-21.4	-19.8	-20.4	-18.6	-21.0
$\delta^{18}O_{H2O}(\%)$	-10.5	-	-11.4	-	-10.9
$\delta^2 H_{H2O}$ (‰)	-61.2	-	-68.4	-	-63.8

Borehole aqueous chemistry, isotope values, and flow rates in the Soudan Mine 27th level West Drift.

"-" not determined.

^a Data from (Sheik et al., 2021), sampling from 2004-2013.

^b \sum (CH₃)₂NH samples were collected in April of 2021 for DDH951.

Anions were measured in triplicate on a Metrohm 850 Professional ion chromatograph using a 3.0 mM sodium carbonate mobile phase and a 250 mm long, 4 mm internal diameter A Supp 5 column and guard. All anion measurements had a relative standard deviation less than 2%. Major cations were measured in triplicate on a Thermo Scientific iCAP 6500 ICP-OES using a Mira Mist PEEK nebulizer. Yttrium was used as a standard to monitor internal consistency. All major cation measurements had a relative standard deviation less than 5%.

3.3. Geochemical modeling

Thermodynamic calculations were performed to assess the affinity for potential chemical reactions occurring in the mine waters. The affinity, as expressed by the Gibbs Free Energy of a given reaction (ΔG), determines which reactions are exergonic and thus, reflect potential metabolic pathways for microorganisms. In this study, the ΔG values of candidate metabolic reactions were assessed for each of three legacy boreholes using measured aqueous abundances and calculated activity coefficients, as described below. The sign of each ΔG value (in kJ/mole units) can be used to determine whether a chemical reaction can proceed spontaneously. If a ΔG value is negative, the forward reaction is favorable (i.e., exergonic). A ΔG value of zero, or near-zero, identifies a reaction at or near equilibrium.

Measured and dissolved volatile abundances, and water and gas flow rates, were used to calculate dissolved concentrations of volatiles in fluids, for use in ΔG calculations. The legacy boreholes sampled at the 27th level west drift are characterized by fluid and gas geochemistry that is relatively stable on the timescale of years (Schuler et al., 2022); therefore, ΔG calculations combine several measurements from multiple sampling trips and boreholes. For example, the abundance of $\sum CO_{2(aq)}$ was not determined for samples collected in February 2019, so an average of the $\sum CO_{2(aq)}$

abundance measured at DDH932 and DDH951 in November 2019 was assumed for DDH944. Furthermore, water and gas flow rates were not determined at DDH932, so an average of the flow rates measured at DDH944 and DDH951 was used.

The ratio of gas flow rate/water flow rate at each borehole was used to calculate the concentration of dissolved volatiles that would have been in solution when fluids and gases were subject to an estimated ~100 bars lithostatic pressure at depth in the reservoir (**Table S1**). This assumption generates a maximum estimate of aqueous gas abundances. If actual reservoir pressures were lower than our estimate, aqueous gas abundances may be lower, and ΔG values would shift accordingly.

Using measured and assumed dissolved concentrations, the activities of reactants and products involved in studied microbial reactions were calculated. Due to high ionic strengths of the Soudan brines, a semi-empirical base Pitzer/Harvie–Møller–Weare (HMW) activity model was used to calculate the activity coefficients of dissolved aqueous species. Calculated activities were compared to standard state equilibrium values to derive thermodynamic affinity or disequilibria for a given reaction (see **SI** for additional details).

4. Results

4.1. Temperature, conductivity, pH, and flow rates

The brines flowing from boreholes DDH932, DDH951, and DDH944 are low temperature (11.5-12.1 °C), high conductivity (121.3-174.4 mS) fluids (**Table 1**) that are 3-5 times more saline than seawater. These salinities are comparable to brines emitted in other deep mines in the Canadian Shield (Frape et al., 1984; Schuler et al., 2022). The pH of the brines increase from 6.1 to 6.9 with distance from east to west down the 27th level west drift. This near-neutral pH range coincides closely with the acid dissociation constants (pKa₁) of the $\sum CO_{2(aq)}$ and $\sum H_2S_{(aq)}$ ($H_2S_{(aq)} + HS^-_{(aq)}$) systems. Thus, pH exerts an important influ-

DDH944

Table 2 Borehole gas chemistry and isotope values in the Soudan Mine 27th level West Drift.					
Borehole # Date	DDH932 (02.26.19)	DDH932 (11.15.19)	DDH951 (02.26.19)	DDH951 (11.15.19)	
CH4 (%)	83.0	81.1	72.6	69.3	

Date	(02.26.19)	(11.15.19)	(02.26.19)	(11.15.19)	(02.26.19)
$\begin{array}{c} CH_4 \ (\%) \\ C_2H_6 \ (\%) \\ C_3H_8 \ (\%) \\ i-C_4H_{10} \ (\%) \\ n-C_4H_{10} \ (\%) \end{array}$	$\begin{array}{c} 83.0 \\ 1.95 \\ 0.115 \\ 6.00 \times 10^{-3} \\ 1.00 \times 10^{-2} \end{array}$	$\begin{array}{c} 81.1 \\ 1.94 \\ 0.118 \\ 7.00 \times 10^{-3} \\ 1.10 \times 10^{-2} \end{array}$	$72.6 \\ 1.36 \\ 9.00 \times 10^{-2} \\ 5.00 \times 10^{-3} \\ 6.00 \times 10^{-3}$	$\begin{array}{c} 69.3 \\ 1.44 \\ 9.80 \times 10^{-2} \\ 7.00 \times 10^{-3} \\ 1.00 \times 10^{-2} \end{array}$	76.1 1.60 - -
N ₂ (%) H ₂ (%) CO ₂ (%) He (%) Ar (%) O ₂ (%)	10.8 <0.05 0.156 0.351 0.319 0.708	11.9 <0.05 - 0.365 0.333 0.963	24.6 <0.05 0.049 0.966 0.477 0.779	23.2 <0.05 - 0.984 0.509 0.511	16.4 0.306 0.113 0.839 0.369 0.628
$\begin{array}{l} \delta^{13} C_{CH4} \ (\%_{0}) \\ \delta^{13} C_{C2H6} \ (\%_{0}) \\ \delta^{13} C_{C3H8} \ (\%_{0}) \\ \delta^{13} C_{nC4H10} \ (\%_{0}) \\ \delta^{13} C_{CO2(g)} \ (\%_{0}) \end{array}$	-42.5 -36.2 -35.0 -33.8 -28.1	-42.1 -38.0 -34.9 -33.8 -	-43.8 -37.5 -36.8 -35.3 -27.0	-43.9 -37.7 -36.7 -35.3 -	-42.9 -36.7 -35.1 -33.7 -27.7
$\delta^{2} H_{CH4} (\%)$ $\delta^{2} H_{C2H6} (\%)$ $\Delta^{13} CH_{3} D (\%)$	-417 -319	-419 -322 4.2	-390 -308 -	-416 -320 4.1	-408 -316 -

"-" not determined.

ence on the dominant inorganic carbon and sulfide species at each borehole. Water flow rates range from 1.1-3.4 mL/s with an uncertainty (2σ) of 0.4 mL/s, while gas flow rates range from 0.18-0.36 mL/s with an uncertainty (2σ) of 0.16 mL/s (**Table 1**).

4.2. Alkanes and inorganic gases

The gas emitted from DDH932, DDH951, and DDH944 is dominated by CH₄, making up 69.3% to 83.0% of the total gas volume. The volume percent of C₂H₆ ranged from 1.36% to 1.95%, along with <1% of C₃H₈, *i*-C₄H₁₀, and *n*-C₄H₁₀ (**Table 2**). These alkane abundances are similar to those emitted from other mines in the Canadian, Fennoscandian, and South African Shields (Sherwood Lollar et al., 1993b; Ward et al., 2004). There is no trend in CH₄ abundance with location along the west drift. However, there are small, ~2-3% decreases in the relative abundance of CH₄ balanced by slight increases of C₂-C₄ alkanes in November 2019, compared to samples collected in February 2019. Gas compositions determined in this study are similar to those measured in 2006 at borehole DDH942, located west of DDH944 on the same level of the Soudan mine, as reported in Sheik et al. (2021).

The second most abundant gas emitted is N₂, which comprises 10.8% to 24.6% of the total gas volume. Taken together, CH₄ and N₂ account for >95% of the total volume once gas budgets are normalized to 100%. Measured $CO_{2(g)}$ ranges from 0.049% to 0.156%. Free molecular H₂ was 0.306% in DDH944 but was below the analytical detection limit (<0.05%) in DDH932 and DDH951. H₂ was also below detection in DDH942 (Sheik et al., 2021). Precambrian Shield gases are known to exhibit wide variability in H₂ content, ranging from below detection to >10% by volume (Li et al., 2016). The abundance of Ar and He ranged from 0.319% to 0.509% and 0.351% to 0.984%, respectively. Any O2 entrained in samples is assumed to be atmospheric contamination due to anoxic conditions of the fluids (Schuler et al., 2022). Measured O₂ ranged from 0.511% to 0.963% by volume. Gas volume percent utilized in thermodynamic models were recalculated to exclude O₂ and corresponding atmospheric-derived N₂ and Ar.

4.3. Aqueous species

Dissolved \sum CO_{2(aq)} concentrations of 0.314 mmol/kg and 0.307 mmol/kg were determined in DDH932 and DDH951, re-

spectively. Abundances for $\sum NH_4^+{}_{(aq)}$ for February 2019 samples ranged from 32.4 µmol/kg to 39.5 µmol/kg. However, borehole DDH932 increased by 42% to 49.6 μ mol/kg Σ NH₄⁺_(aq) in November 2019, while DDH951 experienced a 36% decrease to 20.7 μ mol/kg Σ NH₄⁺(aq) over the same timeframe. These fluctuations exceed analytical uncertainty, indicating that $\sum NH_4^+_{(aq)}$ abundances shifted over 9 months. The concentration of DMA was 41.1 nmol/kg in DDH951, while MMA and TMA were below detection, <100 nmol/kg and <10 nmol/kg, respectively. Aqueous Σ H₂S abundances were too low to be directly measured. Rather, HS^- activity was estimated to be 10^{-11} by assuming mineralfluid equilibrium with a hematite-pyrite-chamosite mineral buffer assemblage at 12 °C, based on observations of these minerals in exposed bedrock and legacy cores matched to the boreholes (Fig. S1). This mineral assemblage would buffer fluid pH to a value slightly below 7, in good agreement with our pH measurements ranging from 6.0-6.9 (Table 1).

4.4. Solvent extractable organics

A variety of organic compounds were identified in the solvent extracts including hydrocarbons, fatty acids (FA), and alcohols. The hydrocarbons were dominated by alkenes with only trace amounts of *n*-alkanes present. Fatty acids and trace amounts of alcohols were identified by their trimethylsilyl (TMS) derivatives within the derivatized samples. Trimethylsilyl FA n-C_{16:0} and n-C_{18:0} are the most abundant FA, and the saturated FA show a strong even-over-odd preference in all samples.

4.5. Water, carbon, and hydrogen isotopes

Water isotope values are similar across the three boreholes, ranging from -11.4% to -10.5% for $\delta^{18}O_{H2O}$ and -68.4% to -61.2% for $\delta^{2}H_{H2O}$. These values have been stable at these boreholes over the past several years (Schuler et al., 2022).

Consistent $\delta^{13}C_{CH4}$ values are observed across the three boreholes, ranging from -43.9% to -42.1%, while longer chain alkane $\delta^{13}C$ values range from -38.0% to -36.2% for C_2H_6 , -36.8% to -34.9% for C_3H_8 , and -35.3% to -33.7% for n- C_4H_{10} . Hydrocarbon stable carbon isotope values determined on February 2019 samples are indistinguishable from those collected in November 2019 except for C_2H_6 in DDH932, which shifts by -1.8% in

November relative to February. Values of $\delta^2 H_{CH4}$ and $\delta^2 H_{C2H6}$ for DDH932 were similar during both sampling trips, ranging from -419% to -417% and -322% and -319%, respectively. Values of $\delta^2 H_{CH4}$ and $\delta^2 H_{C2H6}$ for DDH944 and DDH951 in November 2019 were also similar at -408% and -316%, and -416% and -320%, respectively. Gases collected in February 2019 at DDH951 had $\delta^2 H_{CH4}$ and $\delta^2 H_{C2H6}$ values of -390% and -308%. Measurements of $\delta^{13}C_{\sum CO2(aq)}$ are similar across the three boreholes, ranging from -21.4% to -20.4% in February 2019 and -19.8% to -18.6% in November 2019. Values for $\delta^{13}C_{CO2(g)}$ range from -28.1% to -27.0%.

Values of Δ^{13} CH₃D for DDH932 and DDH951 are 4.2‰ and 4.1‰, respectively. Modeled equilibrium temperatures of Δ^{13} CH₃D are based on the equation:

$$\Delta^{13} \text{CH}_3 \text{D}(\text{T}) = -0.11006 \left(\frac{1000}{T}\right)^3 + 1.04151 \left(\frac{1000}{T}\right)^2 - 0.55235 \left(\frac{1000}{T}\right)$$
(3)

where T is temperature in Kelvin. This equation is derived from fundamental vibrational frequencies calculated using density functional theory (Whitehill et al., 2017). The modeled equilibrium temperatures of CH₄ in boreholes DDH932 and DDH951 are 84 °C and 89 °C, respectively.

5. Discussion

5.1. Isolation of the west drift brine

Deep crystalline bedrock fluids in Precambrian shield settings are often isolated from meteoric surficial waters, as indicated by $\delta^{18}O_{H20}$ and δ^2H_{H20} values that do not align with typical global and local meteoric water trends (Frape and Fritz, 1982) (**Fig. 2**.). The causes of depletions in ¹⁸O_{H20} or enrichments in ²H_{H20} are not fully understood. Several hypotheses have been suggested to explain these trends, including isotopic exchange during the formation of secondary clays and equilibration with silicate minerals and/or gases (e.g., CO₂ and H₂) (Frape and Fritz, 1982).

Several brine reservoirs hosted in Precambrian bedrock have $\delta^{18}O_{H2O}$ and δ^2H_{H2O} values that trend toward the Global Meteoric Water Line (GMWL) (Fig. 2), indicative of mixing between fresh meteoric and highly saline endmembers. At Soudan, meteoric water incursion has been identified in borehole fluids in the east drift on the 27th level (Schuler et al., 2022). These east drift fluids are relatively less saline than those on the west drift, have $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ values that fall near the GMWL (orange circles; Fig. 2), and reflect mixtures of meteoric water and saline brine. In contrast, the west drift brines presented here are characterized by higher salinities and $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ values that are more offset from the local meteoric water line (orange squares; Fig. 2). A previous study demonstrates that higher salinity fluids at Soudan correspond to larger offsets in $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ from the GMWL (Schuler et al., 2022). Similar trends between salinity and water isotopes are also observed in other brines hosted in the Precambrian Canadian Shield (Warr et al., 2021). The deviation from the GMWL $\delta^{18}O_{\rm H2O}$ and $\delta^2H_{\rm H2O}$ trendline in Soudan Mine fluids is likely driven by ^{18}O depletion rather than 2H enrichment due to the absence of abundant H₂ in the boreholes. These results demonstrate that the DDH932, DDH951, and DDH944 brines contain more saline endmember and have experienced little mixing with surficial water sources. We infer that nutrients, carbon, and energy are in limited supply in our fluids relative to reservoirs with more connection to the surface hydrologic system.



Fig. 2. Values of $\delta^{18}O_{H20}$ and δ^2H_{H20} in Precambrian bedrock brines compared to those of the Global Meteoric Water Line (GMWL; solid black line) and Minnesota's Local Meteoric Water Line (LMWL) from (Kendall and Coplen, 2001; dashed gray line). Water isotope data for Soudan are from this study (27th level west drift) and studies from 2009-2011 (27th level west and east drift) (Schuler et al., 2022). Other data are from Kidd Creek (Li et al., 2016), Yellowknife, Thompson, and Sudbury (Frape et al., 1984), in the Canadian Shield and Beatrix and Tau Tona (Simkus et al., 2016) from the South African Shield. Multicolor dashed lines represent the line of best fit for each set of water isotopes. Error bars are smaller than plotted symbols. (For interpretation of the colors in the figure(s), the reader is referred to the web version of this article.)

5.2. Sources of energy for the deep continental biosphere

The anaerobic oxidation of CH₄ (AOM) and longer-chain alkanes has been observed in diverse natural settings, including hydrocarbon seep sediments, hydrothermal vents, and other mineaccessible Precambrian shield reservoirs (Bose et al., 2013; Teske et al., 2002; Magnabosco et al., 2018). High CH₄, C₂H₆, and C₃H₈ at Soudan suggest that alkane oxidation by alternative electron acceptors may represent a significant potential energy source in this energy-limited landscape. To test this hypothesis, the thermodynamic affinity of 11 reactions were determined, including oxidation of alkanes by SO_4^{2-} and NO_3^{-} , the reduction of SO_4^{2-} , NO_3^{-} , and Fe_2O_3 by H_2 , and methanogenesis via the reduction of CO₂ by H₂ and via the breakdown of DMA (Table 3). We have not included TMA or MMA in these calculations, as both chemical species were below analytical detection limits. However, microorganisms at Soudan carry genes for the utilization of both TMA and MMA, indicating that both can be used for methanogenesis (Sheik et al., 2021).

A negative ΔG value in kJ/mole indicates that a reaction can proceed to the right as written, and therefore represents a potential microbial energy source (**Fig. 3**.). However, this standard approach would not directly account for reactant limitations in the Soudan fluids. We therefore take the calculation a step further and use the concentration of the limiting reactant (**Table 3**) to also report ΔG values for each reaction in units of J/kg_{fluid} (**Fig. 4.**, see details in **SI, Table S2**).

The oxidation of CH₄ by SO₄²⁻ at Soudan represents one of the least negative ΔG values in kJ/mole (**Fig. 3**); however, it has the greatest potential energy source per mass fluid in the brines of all reactions modeled, with ΔG ranging from -111 to -95.5 J/kg_{fluid} (**Fig. 4a**). The oxidation of CH₄, C₂H₆, and C₃H₈ by NO₃⁻ and oxidation of C₂H₆ and C₃H₈ by SO₄²⁻ are also energetically fa-

Table 3

Candidate metabolic reactions.			
Reaction #	Reaction	Limiting reactant	
1	$\mathrm{CH_4} + \mathrm{SO_4}^{2-} \rightarrow \mathrm{HCO_3}^- + \mathrm{HS}^- + \mathrm{H_2O}$	SO4 ²⁻	
2	${\rm C_2H_6} + 1.75{\rm SO_4}^{2-} \rightarrow 2{\rm HCO_3}^- + 1.75{\rm HS}^- + {\rm H_2O} + 0.25{\rm H^+}$	C ₂ H ₆	
3	${\rm C_3H_8} + 2.5{\rm SO_4}^{2-} \rightarrow 3{\rm HCO_3}^- + 2.5{\rm HS}^- + {\rm H_2O} + 0.5{\rm H^+}$	C ₃ H ₈	
4	$\rm CH_4 + \rm NO_3^- + \rm H^+ \rightarrow \rm HCO_3^- + \rm NH_4^+$	NO ₃ ⁻	
5	$\text{C}_{2}\text{H}_{6} + 1.75\text{NO}_{3}^{-} + 0.75\text{H}_{2}\text{O} + 1.5\text{H}^{+} \rightarrow 2\text{HCO}_{3}^{-} + 1.75\text{NH}_{4}^{+}$	NO ₃ ⁻	
6	$\rm C_{3}H_{8} + 2.5NO_{3}^{-} + 1.5H_{2}O + 2H^{+} \rightarrow \rm 3HCO_{3}^{-} + 2.5NH_{4}^{+}$	NO3 ⁻ (932), C3H8 (951)	
7	$\mathrm{SO_4^{2-}} + 4\mathrm{H_2} + \mathrm{H^+} \rightarrow \mathrm{HS^-} + 4\mathrm{H_2O}$	H ₂	
8	$\mathrm{NO_3}^- + 4\mathrm{H_2} + 2\mathrm{H^+} \rightarrow \mathrm{NH_4}^+ + 3\mathrm{H_2O}$	H ₂	
9	$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	H ₂	
10	$Fe_2O_3 + H_2 + 4H^+ \rightarrow 2Fe^{2+} + 3H_2O$	H ₂	
11	$(\text{CH}_3)_2\text{NH}_2^+ + 1.5\text{H}_2\text{O} \rightarrow 1.5\text{CH}_4 + 0.5\text{HCO}_3^- + \text{NH}_4^+ + 0.5\text{H}^+$	$(CH_3)_2 NH_2^+$	



Fig. 3. Calculated Gibbs Free Energy (ΔG) available in kJ/mole for reactions in Table 3. Reactions plotted include the oxidation of CH₄, C_2H_6 , and C_3H_8 by SO₄²⁻ via reactions 1, 2, and 3, respectively (orange tones), the oxidation of CH₄, C_2H_6 , and C_3H_8 by NO₃⁻ via reactions 4, 5, and 6, respectively (blue tones), reactions involving the reduction of SO₄²⁻ (yellow), NO₃⁻ (teal), CO₂ (green), and Fe₂O₃ (dark gray) by H₂ via reactions 7, 8, 9, and 10 respectively, and methanogenesis from DMA via reaction 11 (light gray).



Fig. 4. Calculated Gibbs Free Energy (ΔG) available in 1 kg of fluid determined for (a) reactions involving the oxidation of CH₄, C₂H₆, and C₃H₈ by SO₄²⁻ via Table 3 reactions 1, 2, and 3, respectively (orange tones) and NO₃⁻, via Table 3 reactions 4, 5, and 6, respectively (blue tones); and (b) reactions involving the reduction of SO₄²⁻ (yellow), NO₃⁻ (teal), CO₂ (green), and Fe₂O₃ (gray) by H₂ via Table 3 reactions 7, 8, 9, and 10 respectively. Note that the y-axis scale differs in panels (a) and (b). Methanogenesis from DMA via reaction 11 is not shown because ΔG values for this reaction in J/kg_{fluid} units are near-zero.

vorable and collectively range from -19.9 to -1.07 J/kg_{fluid}. Using previously created shotgun metagenome assemblies (Sheik et al., 2021), organisms and genes indicative of CH₄ and alkane degradation were explored. There are several potential routes for biological anaerobic CH₄ oxidation (AOM). However, no anaerobic methanotrophic (ANME) Archaea or Methylomirabilota (NC10 bacteria) were detected in the metagenomes. Several ANME archaea can oxidize CH₄ with SO_4^{2-} or NO_3^{-} , while NC10 bacteria grow in anaerobic environments but use O₂ produced intracellularly from NO_3^- reduction to oxidize methane (see Chadwick et al., 2022) and references within). Additionally, alkyl succinate synthase genes that anaerobic bacteria can use to degrade short to medium chain alkanes were not detected. Given that C₂-C₄ alkane concentrations are similar to those in other Precambrian Shield environments, it is likely that microorganisms may not prefer to metabolize them or that consumption does not outweigh production.

The potential energy per mass fluid for reactions involving reduction by H₂ is modeled in DDH944 (**Fig. 4b**), but not for DDH932 and DDH951, where H₂ was below detection. The potential energies for reduction of SO₄^{2–}, NO₃⁻, and Fe₂O₃ by H₂ are -1.00, -3.42, and -0.810 J/kg_{fluid} respectively. The observation of free molecular H₂ only at DDH944 contrasts with the strong chemical similarity that is otherwise observed across the three boreholes. Two hypotheses that might account for the presence of H₂ in DDH944 and absence in DDH932 and DDH951 are (1) microbes are consuming H₂ at a rate exceeding its replenishment rate in DDH932, and DDH951 (Sheik et al., 2021) and/or (2) H₂ is independently sourced to DDH944 through a fracture network that is isolated from the other boreholes located further east along the drift. Given the similarity in other chemistry, hypothesis (1) is the most likely explanation.

The ΔG values that we determined for Soudan, in kJ/mole units, for alkane oxidation by SO₄²⁻ (reactions 1-3, Table 3) and SO₄²⁻ reduction by H₂ (reaction 7, Table 3) are similar to analogous ΔG values calculated at the Kloof mine in the Witwatersrand Basin of South Africa (Kieft et al., 2005). In contrast, reactions involving CH₄ oxidation by SO₄²⁻ and NO₃⁻ (reactions 1 and 4, Table 3) and SO₄²⁻ and NO₃⁻ reduction by H₂ (reactions 7 and 8, Table 3) have greater energy potential at Soudan compared to the Sanford Underground Research Facility (SURF) in South Dakota, USA (Osburn et al., 2014). Kloof and SURF are also hosted in Precambrian aged bedrock and emit alkanes and fluids from legacy boreholes, similar to the Soudan mine.

Values of ΔG as low as -10 kJ/mol have been suggested to support microbial metabolism (Hoehler, 2004). All calculated ΔG values in this study are more negative than this threshold, indicating that minimum energy requirements are met in the Soudan fluids (**Fig. 3**). More negative ΔG values imply that organisms can mediate these reactions more favorably. However, thermodynamics alone does not dictate substrate utilization but rather the energy demand to process these reactions. The high potential energy for specific reactions could also be evidence of a lack of active metabolic pathways that utilize them. The integration of chemical (e.g., thermodynamic) and biological (e.g., metagenomic and culture) assessments is key to identifying which in situ microbial processes are actively occurring in fluids.

Methanogenesis may be a pillar in the carbon cycle along with other non-biological processes that produce alkanes in the Soudan Mine. The calculated ΔG value for methanogenesis by CO_2 reduction with H_2 via reaction 9 in Table 3 is exergonic $(\Delta G = -87.8 \text{ kJ/mol})$ (Fig. 3). Genomics assessments, however, have not identified hydrogenotrophic methanogens that would mediate this reaction (Sheik et al., 2021). Instead, methanogens that utilize methylated substrates were detected in the Soudan fluids by Sheik and colleagues. Methyl-based methanogenesis produces CH_4 via the breakdown of a methyl group $(-CH_3)$ in an organic compound, and thus differs fundamentally from hydrogenotrophic methanogenesis which involves the reduction of inorganic carbon (e.g., CO₂) by H₂. It has also been suggested that methyl-based methanogens may actively cycle H₂, as observed in marine sediment experiments (Finke et al., 2007). While methylamines (MMA, DMA, and TMA) have been guantified in several marine environments (Zhuang et al., 2017; Cree et al., 2018; Sun et al., 2019) and have been utilized as substrates for methanogenesis in isolated cultures from deep-sea coal beds, terrestrial shales, and shallow marine sediments (Finke et al., 2007; Vigneron et al., 2015; Daly et al., 2016; Trembath-Reichert et al., 2017), this study is the first to identify DMA in natural fluids hosted in Precambrian bedrock. It is proposed that glycine betaine, a common osmolyte for halobacteria (Sleator and Hill, 2001), may be converted to TMA and subsequently demethylated to DMA and MMA to produce CH4 in Soudan's fluids (Sheik et al., 2021). The presence of DMA in DDH951 provides support for this suggested pathway. TMA may be demethylated rapidly through this process to concentrations below detection (<10 nmol/kg), producing quantifiable DMA similar to that found in methanogenic cultures when using TMA as a substrate (Watkins et al., 2014). The reaction for methanogenesis via the breakdown of DMA (reaction 11, Table 3) is exergonic ($\Delta G = -142 \text{ kJ/mol}$) (Fig. 3) but with very low available energy per mass fluid (-0.00635 J/kg_{fluid}). These findings insinuate that methyl-based methanogenesis is favorable and the low energy available per mass of fluid may be a manifestation of rapid consumption of DMA as a reactant while concordantly producing biological CH₄.

5.3. The origin of alkanes

Identifying the origin of CH_4 and C_{2+} n-alkanes in the Soudan mine gas is a complex problem due to microorganisms that can actively manipulate the C cycle. In turn, the kinetics for spontaneous $\sum CO_{2(aq)}$ reduction via abiotic reactions are sluggish or inhibited in low-temperature environments (<200 °C) (McCollom, 2016). Alkane abiogenesis is proposed within deep crustal gasrich reservoirs in several mines in Precambrian Shield settings (Sherwood Lollar et al., 2002; Kietäväinen et al., 2017), and parallels are drawn to their abiotic synthesis in hydrothermal vents (McDermott et al., 2015). Stable isotope measurements, such as $\delta^{13}C_{CH4}$, are a classic approach to determining the origin and cycling of carbon species in natural systems. One difference between the continental and marine environment is that presumed abiotic $\delta^{13}C_{CH4}$ values are more ^{13}C -depleted in continental settings (approximately -40% to -30%) compared to seafloor hydrothermal fluids (approximately -20% to -10%) (McCollom and Seewald, 2007). Values of $\delta^{13}C_{CH4}$ at Soudan of -43.9% to -42.1% are \sim 5-10[%]0 more depleted than proposed abiotic signatures at Kidd Creek (Sherwood Lollar et al., 2002) (Fig. 5). In general, values of



Fig. 5. The ratio of C₁/C₂₊ alkanes plotted against $\delta^{13}C_{CH4}$ in Precambrian Shield brines. Dashed boxes represent typical microbial and non-microbial values from (McCollom and Seewald, 2007). Colored ellipses encompass the cluster of data points from each site. Data are from the Soudan mine (this study), the Kidd Creek (Sherwood Lollar et al., 2002), Yellowknife, and Thompson mines (Sherwood Lollar et al., 1993a) in the Canadian Shield, and the Beatrix, Evander, and Kloof mines in the South African Shield (Ward et al., 2004). Error bars are smaller than plotted symbols.

 $δ^{13}C_{CH4}$ produced via active methanogenesis are significantly depleted relative to CH₄ produced via thermogenic and abiotic processes (Sherwood Lollar et al., 1993b; Ward et al., 2004) (**Fig. 5**). Microbial $δ^{13}C_{CH4}$ values are typically <-50‰ (Rice and Claypool, 1981) and are directly influenced by the $δ^{13}C$ of the source of carbon. Thermogenic alkanes exhibit a wide range of $δ^{13}C$ values, which can also be attributed to differences in source materials and thermal maturity (Rooney et al., 1995).

Trends in δ^{13} C values can also be assessed for longer chain alkanes. C₁-C₄ alkanes produced through thermogenic processes often show a positive enrichment of δ^{13} C with increasing carbon number due to the preferential primary cleaving of ¹²C and ¹H bonds forming CH₄ during organic pyrolysis (Clayton, 1991). This enrichment trend is observed in Soudan's gases (**Fig. S2**). In contrast, isotopic 'reversals' between $\delta^{13}C_{CH4}$ and more depleted $\delta^{13}C_{C2+}$ are postulated to be produced abiotically (Sherwood Lollar et al., 2002). However, several laboratory-based abiotic synthesis studies produced alkanes with isotope enrichment trends that mimic those classically associated with thermogenesis (McCollom, 2013 and refs. therein).

Further, abiotic C₁-C₃ alkanes in some natural seafloor hydrothermal fluids also show positive enrichment of δ^{13} C values with increasing carbon number (McDermott et al., 2015). Finally, enriched $\delta^{13}C_{CH4}$ compared to depleted $\delta^{13}C_{C2+}$ has also been observed in thermogenic settings due to the possible mixing of mature and immature gas in open systems with multiple sources of organic material (Fiebig et al., 2019). Isotope trends alone are therefore insufficient to identify non-biological organic synthesis pathways unequivocally. Further study of natural abiotic synthesis of alkanes is needed, particularly in continental environments.

In systems where methanogenesis is a dominant contributor to the CH₄ pool, high C_1/C_{2+} ratios are observed due to the high abundance of microbial CH₄ relative to C_{2+} alkanes (Rooney et al., 1995). Although the biological production of longer-chained alkanes has been identified in marine sediments (Hinrichs et al., 2006), the significance of biological C_{2+} production in deep continental environments remains unknown. Soudan's C_1/C_{2+} values



Fig. 6. Modeled Δ^{13} CH₃D formation temperature. The solid black line is derived from fundamental vibrational frequencies calculated using density functional theory (Whitehill et al., 2017).

are lower than those in settings that are thought to reflect purely microbial-produced gas. However, the C_1/C_{2+} and $\delta^{13}C_{CH4}$ values are trending away from typical non-microbial ranges, towards the microbial field in Fig. 5. These values reflect a mixture of predominantly non-microbial CH₄ with lesser degrees of biological CH₄ at Soudan. This conclusion closely aligns with gases collected in the Evander mine in the South African Shield (Ward et al., 2004) (**Fig. 5**).

It is plausible that methyl-based methanogenesis may be the biological source of CH₄, mixing with a non-microbial source and depleting bulk $\delta^{13}C_{CH4}$ at Soudan. Other processes such as the anaerobic oxidation of methane (AOM) could concurrently influence isotopic and abundance values, enriching the $\delta^{13}C_{CH4}$ values of the residual CH₄ pool. However, if AOM were a dominant control on CH₄ abundance, one would expect Soudan's $\delta^{13}C_{CH4}$ values (-43.9‰ to -42.1‰) to be more enriched than typical non-microbial values (-40‰ to -30‰) due to the residual enrichment of $\delta^{13}C_{CH4}$ through methanotrophy.

Along with traditional δ^{13} C isotope signatures, rare doubly sub-stituted 'clumped' isotopes of CH₄ (i.e., ¹³CH₃D) provide valuable information that may be used to determine CH₄ formation temperatures or identify microbial influences (Ono et al., 2014; Stolper et al., 2014; Wang et al., 2015). During the formation of CH_4 , Δ^{13} CH₃D (equation (2)) can equilibrate at the formation temperature (**Fig. 6**). Provided that Δ^{13} CH₃D does not undergo further isotopic exchange after post-formation changes in environmental temperature (horizontal arrow, Fig. 7), the 'clumped' isotope may be used as a geothermometer (Stolper et al., 2014). However, microbial manipulation of CH₄ can shift Δ^{13} CH₃D values to a state of disequilibrium, as demonstrated by cultures and natural samples (Wang et al., 2015; Ono et al., 2021). Natural biological 'clumped' CH₄ values have been observed to equilibrate at low environmental temperatures in some cases, as seen in the biodegradation of oil by methanogens in the Gulf of Mexico (Stolper et al., 2014) (Fig. 7). In other cases, several methanogenic cultures (H₂ and methylbased) and natural samples show 'de-clumped' Δ^{13} CH₃D signals, with apparent extreme high temperatures of 200 to >500 °C (upward arrow, Fig. 7) (Wang et al., 2015; Gruen et al., 2018). Abiotic processes have also been postulated to slightly lower $\Delta^{13}CH_3D$ values in continental settings (Young et al., 2017), although not to the extent of methanogenesis. Methanotrophic cultures have also been observed to reverse this 'de-clumping' effect, resulting in enriched Δ^{13} CH₃D values on the residual CH₄ pool and shifting modeled temperatures lower (downward arrow, Fig. 7), possibly lower than environmental temperatures (Ono et al., 2021). Soudan Δ^{13} CH₃D values of 4.1-4.2‰ point to formation temperatures of 84°C and 89°C (Fig. 6). These formation temperatures are warmer than present-day environmental temperatures of 11.5-12.1 °C (**Fig. 7**), but below the typical threshold of \sim 100 °C needed to form natural gas (Rooney et al., 1995). These temperatures also approach the known upper limit for microbial life (122°C, Takai et al., 2008). The difference between CH₄ formation temperature and environmental temperature may be due to the migration of microbial Δ^{13} CH₃D equilibrated at warmer temperatures at depth. However, a formation temperature of 84-89°C puts the estimated depth of the water source at >6 km, assuming a thermal gradient of 12-14 °C/km (Artemieva and Mooney, 2001), much deeper than the 0.7 km depth of the 27th level of the mine plus the extended 144 m of borehole depth (Fig. 1). Two other hypotheses that would better explain this temperature discrepancy are (1) there is mixing of microbial equilibrated Δ^{13} CH₃D at environmental temperatures (11.5-12.1 °C) and non-microbial Δ^{13} CH₃D equilibrated at higher formation temperatures; or (2) non-microbial Δ^{13} CH₃D equilibrated at higher formation temperatures, environmental temperatures cooled to allow for life to persist, then methanotrophic organisms consumed CH₄, enriching the residual ¹³CH₃D and drawing Δ^{13} CH₃D values closer to equilibrium. Values in Fig. 5 show that mixing of microbial and non-microbial CH₄ is likely, which is consistent with hypothesis (1). Hypothesis (2) is also compelling due to the modeled exergonic reactions involving AOM (Figs. 3 and **4a**). However, Soudan's slightly depleted $\delta^{13}C_{CH4}$ values compared to non-microbial sources suggest that this is not the case.

Distinguishing microbial and thermogenic 'clumped' CH₄ signatures from those that are abiotic remains a difficult task. Soudan's Δ^{13} CH₃D values are similar to those of presumed abiotic CH₄ in Kidd Creek Mine, Ontario (Fig. 7). The sites are chemically distinct (e.g., Kidd Creek contains > 10% H₂, $\delta^{13}C_{CH4}$ values of ~-35% (Sherwood Lollar et al., 2002; Li et al., 2016)) but they nonetheless occupy a similar space in Fig. 7, with a similar modeled temperature for Δ^{13} CH₃D. It is also interesting to note that, although Soudan and Kidd Creek's C_1 - C_4 alkane $\delta^{13}C$ values do not overlap and display distinct trends with increasing carbon number (**Fig. S2**), the $\delta^2 H_{CH4}$ and $\delta^2 H_{C2H6}$ values determined in this study (-419% to -390% and -322% to -308%) are indistinguishable from those measured at Kidd Creek (-419% to -390% and -321% to -299%) (Sherwood Lollar et al., 2002). For abiogenesis to occur, sufficient quantities of H₂ are needed. Unlike Kidd Creek, Soudan lacks significant free H₂, suggesting that abiotic production of C₁-C₄ alkanes is unlikely. Soudan's C₁-C₄ alkanes are therefore likely sourced from a thermogenic origin mixed with a supply of CH₄ from microbial processes.

The trace C_{10+} n-alkanes in Soudan's fluids that are present in the solvent extractable organic fraction are not sourced through thermogenic precursors, but rather from non-thermally altered biological communities as indicated by their odd-over-even preference and non-unimodal distribution (Tissot and Welte, 1984). This conclusion is further supported by the observed prevalence of alkenes over n-alkanes and fatty acid (FA) distributions in the solvent extractable organic fraction (Fig. S3). The prominent n- $C_{16:0}$ and $n-C_{18:0}$ FA, which account for the major FA in all three boreholes, are often the most abundant in many bacteria and eukaryotes (Thurman, 1985). The C_1 - C_4 alkane thermogenic pool at Soudan may thus be derived from an alternative source, such as older high temperature gas generated by pyrolysis of organics, or the migration of gases from an external thermogenic source. Although the current temperature at the 27th level is 11.5-12.1 °C, the thermal history of its bedrock suggests that alteration of organic material would have been possible. Hydrothermal modification of Soudan's BIF at 150 °C to >300 °C is proposed to have occurred post-deposition (>2.5 Ga) with several deformation events occurring afterwards (Thompson, 2015). This temperature range would support pyrolysis of organics that may have been trapped



Fig. 7. Modeled Δ^{13} CH₃D temperature plotted against current environmental temperature. The solid black line represents a 1:1 ratio of modeled versus environmental temperature. Gray arrows represent possible pathways that could influence modeled temperatures as described in the main text. Data shown are from Kidd Creek, Beatrix, Kloof, and Tau Tona (Ward et al., 2004; Wang et al., 2015; Young et al., 2017), Haynesville Shale, Marcellus Shale, Eagle Ford Shale, Potiguar Basin, and Gulf of Mexico (Stolper et al., 2014; Xie et al., 2021). Temperatures from Stolper et al. (2014) are from Δ_{18} , a parameter that uses combined signals of clumped CH₄ isotopologues ¹³CH₃D and ¹²CH₂D₂.

in the bedrock, possibly producing CH₄ and volatile alkanes. Subsequent tectonic events may have also formed conduits for external thermogenic CH₄ to migrate to the Soudan fracture network.

6. Conclusions

Legacy boreholes on Soudan's 27th level west drift expel chemically and isotopically similar brines, indicating that they likely tap one source fluid. Values of $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ that deviate from global and local meteoric water lines indicate that this saline endmember is largely isolated from surficial waters. Therefore, chemotrophic interactions currently drive energy production for subsurface microbial communities. The high abundance of C_1 - C_4 alkanes and negative ΔG values for alkane oxidation by SO_4^{2-} and NO_3^{-} demonstrate that these compounds are viable energy sources. Reactions involving the reduction of oxidized species by H₂ are exergonic in DDH944. The lack of detectable H₂ in DDH951 and DDH932 may be due to its rapid consumption in these boreholes due to the presence of non-methanogenic hydrogen oxidizing microbes (Sheik et al., 2021). Although methanogenesis involving CO2 reduction by H2 is exergonic, an absence of hydrogenotrophic organisms suggests that this pathway is not active and is likely competitively excluded by the low H₂ concentrations. Instead, Sheik and colleagues identify methyl-based methanogens that could utilize methylamines. The observation that DMA methanogenesis is exergonic with very low available energy per mass fluid supports these assertions. Methylamines may thus be a key substrate for the biological production of CH₄ in Soudan. Hydrocarbon abundance ratios and δ^{13} C isotope signatures show that mixing of CH₄ of both microbial and non-microbial origin is likely occurring. Results from doubly substituted 'clumped' CH4 (i.e., ¹³CH₃D) also support mixed sources of CH₄ due to the warm modeled temperatures (84-89 °C) that are well above Soudan's environmental temperatures but below thermogenic production thresholds. Similar Δ^{13} CH₃D values for 'mixed' CH₄ from Soudan compared to presumed abiotic CH₄ from Kidd Creek demonstrates the need for more examinations into 'clumped' CH₄ signals across a diverse set of low temperature, natural continental settings. The abundance of C_{2+} alkanes, coupled to minimal H_2 concentrations in Soudan, point to a likely thermogenic origin, though an abiotic source of hydrocarbons cannot be entirely ruled out. This study quantifies the availability of energy sources to chemotrophic microbes in Soudan Mine in the present day. These findings illuminate a 'hidden' modern deep continental biosphere and set the stage to model past and future habitability controls. These investigations can also aid planetary studies that seek to determine biogeochemical conditions relevant to habitability on other bodies in our solar system by using Earth-based systems as extraterrestrial analogues.

CRediT authorship contribution statement

William S. Dowd: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Christopher J. Schuler: Investigation, Writing – review & editing. Cara M. Santelli: Funding acquisition, Investigation, Project administration, Writing – review & editing. Brandy M. Toner: Funding acquisition, Investigation, Project administration, Writing – review & editing. Cody S. Sheik: Funding acquisition, Investigation, Project administration, Writing – review & editing. Kelden Pehr: Investigation, Writing – review & editing. Jill M. McDermott: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by National Science Foundation Grants #EAR-1813237 and #EAR-1813526, and the NASA Astrobiology Grant #80NSSC18M0094. This material is based upon research supported by the National Aeronautics and Space Administration through the NASA Astrobiology Institute under Cooperative Agreement No. 80NSSC18M0094 issued through the Science Mission Directorate. We thank the Soudan Underground Mine State Park staff for providing access to the field site and assisting with permit applications. We are grateful to mine staff and colleagues from Lehigh University, University of Minnesota Twin Cities, and University of Minnesota Duluth for helping coordinate multiple sampling trips. We thank editor Boswell Wing and two anonymous reviewers for providing helpful feedback that improved this manuscript.

Appendix A. Supplementary material

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.epsl.2022.117720.

References

- Artemieva, I.M., Mooney, W.D., 2001. Thermal thickness and evolution of Precambrian lithosphere: a global study. J. Geophys. Res. 106, 16387–16414. https:// doi.org/10.1029/2000JB900439.
- Bose, A., Rogers, D.R., Adams, M.M., Joye, S.B., Girguis, P.R., 2013. Geomicrobiological linkages between short-chain alkane consumption and sulfate reduction rates in seep sediments. Front. Microbiol. 4, 1–13. https://doi.org/10.3389/fmicb.2013. 00386.
- Chadwick, G.L., Skennerton, C.T., Laso-Pérez, R., Leu, A.O., Speth, D.R., Yu, H., Morgan-Lang, C., Hatzenpichler, R., Goudeau, D., Malmstrom, R., Brazelton, W.J., Woyke, T., Hallam, S.J., Tyson, G.W., Wegener, G., Boetius, A., Orphan, V.J., 2022. Comparative genomics reveals electron transfer and syntrophic mechanisms differentiating methanotrophic and methanogenic archaea. PLoS Biol., 1–70. https:// doi.org/10.1371/journal.pbio.3001508.
- Clayton, C., 1991. Carbon isotope fractionation during natural gas generation from kerogen. Mar. Pet. Geol. 8, 232–240. https://doi.org/10.1016/0264-8172(91) 90010-X.
- Cree, C.H.L., Airs, R., Archer, S.D., Fitzsimons, M.F., 2018. Measurement of methylamines in seawater using solid phase microextraction and gas chromatography. Limnol. Oceanogr., Methods 16, 411–420. https://doi.org/10.1002/lom3.10255.
- Daly, R.A., Borton, M.A., Wilkins, M.J., Hoyt, D.W., Kountz, D.J., Wolfe, R.A., Welch, S.A., Marcus, D.N., Trexler, R.V., Macrae, J.D., Krzycki, J.A., Cole, D.R., Mouser, P.J., Wrighton, K.C., 2016. Microbial metabolisms in a 2.5-km-deep ecosystem created by hydraulic fracturing in shales. Nat. Microbiol. 1, 1–9. https://doi.org/ 10.1038/nmicrobiol.2016.146.
- Fiebig, J., Stefánsson, A., Ricci, A., Tassi, F., Viveiros, F., Silva, C., Lopez, T.M., Schreiber, C., Hofmann, S., Mountain, B.W., 2019. Abiogenesis not required to explain the origin of volcanic-hydrothermal hydrocarbons. Geochem. Perspect. Lett. 11, 23–27. https://doi.org/10.7185/geochemlet.1920.
- Finke, N., Hoehler, T.M., Jørgensen, B.B., 2007. Hydrogen 'leakage' during methanogenesis from methanol and methylamine: implications for anaerobic carbon degradation pathways in aquatic sediments. Environ. Microbiol. 9, 1060–1071. https://doi.org/10.1111/j.1462-2920.2007.01248.x.
- Frape, S.K., Fritz, P., 1982. The chemistry and isotopic composition of saline groundwaters from the Sudbury Basin, Ontario. Can. J. Earth Sci. 19, 645–661. https:// doi.org/10.1139/e82-053.
- Frape, S.K., Fritz, P., McNutt, R.H., 1984. Water-rock interaction and chemistry of groundwaters from the Canadian Shield. Geochim. Cosmochim. Acta 48, 1617–1627. https://doi.org/10.1016/0016-7037(84)90331-4.
- Gruen, D.S., Wang, D.T., Könneke, M., Topçuoğlu, B.D., Stewart, L.C., Goldhammer, T., Holden, J.F., Hinrichs, K.-U., Ono, S., 2018. Experimental investigation on the controls of clumped isotopologue and hydrogen isotope ratios in microbial methane. Geochim. Cosmochim. Acta 237, 339–356. https://doi.org/10.1016/ j.gca.2018.06.029.
- Hinrichs, K.-U., Hayes, J.M., Bach, W., Spivackl, A.J., Hmelo, L.R., Holm, N.G., Johnson, C.G., Sylva, S.P., 2006. Biological formation of ethane and propane in the deep marine subsurface. Proc. Natl. Acad. Sci. 103, 14684–14689. https://doi.org/10. 1073/pnas.0606535103.
- Hoehler, T.M., 2004. Biological energy requirements as quantitative boundary conditions for life in the subsurface. Geobiology 2, 205–215. https://doi.org/10.1111/ j.1472-4677.2004.00033.x.
- Holland, G., Sherwood Lollar, B., Li, L., Lacrampe-Couloume, G., Slater, G.F., Ballentine, C.J., 2013. Deep fracture fluids isolated in the crust since the Precambrian era. Nature 497, 357–360. https://doi.org/10.1038/nature12127.

- Hudak, G.J., Peterson, D.M., Radakovich, A., Pignotta, G., Schwierske, K., Students from the 2010-2013 Precambrian Research Center Geology Field Camp, 2016. Bedrock Geology of the Lake Vermilion/Soudan Underground Mine State Park. Nat. Resour. Res. Institute, Univ. Minnesota Duluth.
- Kendall, C., Coplen, T.B., 2001. Distribution of oxygen-18 and deuteriun in river waters across the United States. Hydrol. Process. 15, 1363–1393. https://doi.org/10. 1002/hyp.217.
- Kieft, T.L., McCuddy, S.M., Onstott, T.C., Davidson, M., Lin, L.-H., Mislowack, B., Pratt, L., Boice, E., Sherwood Lollar, B., Lippmann-Pipke, J., Pfiffner, S.M., Phelps, T.J., Gihring, T., Moser, D., van Heerden, A., 2005. Geochemically generated, energyrich substrates and indigenous microorganisms in deep, ancient groundwater. Geomicrobiol. J. 22, 325–335. https://doi.org/10.1080/01490450500184876.
- Kietäväinen, R., Ahonen, L., Niinikoski, P., Nykänen, H., Kukkonen, I.T., 2017. Abiotic and biotic controls on methane formation down to 2.5 km depth within the Precambrian Fennoscandian Shield. Geochim. Cosmochim. Acta 202, 124–145. https://doi.org/10.1016/j.gca.2016.12.020.
- Lever, M.A., Rogers, K.L., Lloyd, K.G., Overmann, J., Schink, B., Thauer, R.K., Hoehler, T.M., Jørgensen, B.B., 2015. Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. FEMS Microbiol. Rev. 39, 688–728. https://doi.org/10.1093/femsre/fuv020.
- Li, L., Wing, B.A., Bui, T.H., McDermott, J.M., Slater, G.F., Wei, S., Lacrampe-Couloume, G., Sherwood Lollar, B., 2016. Sulfur mass-independent fractionation in subsurface fracture waters indicates a long-standing sulfur cycle in Precambrian rocks. Nat. Commun. 7, 1–9. https://doi.org/10.1038/ncomms13252.
- Magnabosco, C., Timmers, P.H.A., Lau, M.C.Y., Borgonie, G., Linage-Alvarez, B., Kuloyo, O., Alleva, R., Kieft, T.L., Slater, G.F., van Heerden, E., Sherwood Lollar, B., Onstott, T.C., 2018. Fluctuations in populations of subsurface methane oxidizers in coordination with changes in electron acceptor availability. FEMS Microbiol. Ecol. 94, 1–10. https://doi.org/10.1093/femsec/fiy089.
- McCollom, T.M., 2016. Abiotic methane formation during experimental serpentinization of olivine. Proc. Natl. Acad. Sci. 113, 13965–13970. https://doi.org/10.1073/ pnas.1611843113.
- McCollom, T.M., 2013. Laboratory simulations of abiotic hydrocarbon formation in Earth's deep subsurface. Rev. Mineral. Geochem. 75, 467–494. https://doi.org/10. 2138/rmg.2013.75.15.
- McCollom, T.M., Seewald, J.S., 2007. Abiotic synthesis of organic compounds in deepsea hydrothermal environments. Chem. Rev. 107, 382–401. https://doi.org/10. 1021/cr0503660.
- McDermott, J.M., Seewald, J.S., German, C.R., Sylva, S.P., 2015. Pathways for abiotic organic synthesis at submarine hydrothermal fields. Proc. Natl. Acad. Sci. 112, 7668–7672. https://doi.org/10.1073/pnas.1506295112.
- McMahon, S., Parnell, J., 2014. Weighing the deep continental biosphere. FEMS Microbiol. Ecol. 87, 113–120. https://doi.org/10.1111/1574-6941.12196.
- Nurmi, P.A., Kukkonen, I.T., Lahermo, P.W., 1988. Geochemistry and origin of saline groundwaters in the Fennoscandian Shield. Appl. Geochem. 3, 185–203. https:// doi.org/10.1016/0883-2927(88)90007-8.
- Ono, S., Rhim, J.H., Gruen, D.S., Taubner, H., Kölling, M., Wegener, G., 2021. Clumped isotopologue fractionation by microbial cultures performing the anaerobic oxidation of methane. Geochim. Cosmochim. Acta 293, 70–85. https://doi.org/10. 1016/j.gca.2020.10.015.
- Ono, S., Wang, D.T., Gruen, D.S., Sherwood Lollar, B., Zahniser, M.S., Mcmanus, B.J., Nelson, D.D., 2014. Measurement of a doubly substituted methane isotopologue, ¹³CH₃D, by tunable infrared laser direct absorption spectroscopy. Anal. Chem. 86, 6487–6494. https://doi.org/10.1021/ac5010579.
- Onstott, T.C., Moser, D.P., Pfiffner, S.M., Fredrickson, J.K., Brockman, F.J., Phelps, T.J., White, D.C., Peacock, A., Balkwill, D., Hoover, R., Krumholz, L.R., Borscik, M., Kieft, T.L., Wilson, R., 2003. Indigenous and contaminant microbes in ultradeep mines. Environ. Microbiol. 5, 1168–1191. https://doi.org/10.1046/j.1462-2920.2003.00512.x.
- Osburn, M.R., LaRowe, D.E., Momper, L.M., Amend, J.P., 2014. Chemolithotrophy in the continental deep subsurface: Sanford underground research facility (SURF), USA. Front. Microbiol. 5, 1–14. https://doi.org/10.3389/fmicb.2014.00610.
- Reeves, E.P., Fiebig, J., 2020. Abiotic synthesis of methane and organic compounds in Earth's lithosphere. Elements 16, 25–31. https://doi.org/10.2138/gselements.16. 1.25.
- Reeves, E.P., Seewald, J.S., Sylva, S.P., 2012. Hydrogen isotope exchange between n-alkanes and water under hydrothermal conditions. Geochim. Cosmochim. Acta 77, 582–599. https://doi.org/10.1016/j.gca.2011.10.008.
- Rice, D.D., Claypool, G.E., 1981. Generation, accumulation, and resource potential of biogenic gas. Am. Assoc. Pet. Geol. Bull. 65, 5–25. https://doi.org/10.1306/ 2F919765-16CE-11D7-8645000102C1865D.
- Rooney, M.A., Claypool, G.E., Moses Chung, H., 1995. Modeling thermogenic gas generation using carbon isotope ratios of natural gas hydrocarbons. Chem. Geol. 126, 219–232. https://doi.org/10.1016/0009-2541(95)00119-0.
- Schuler, C.J., Briscoe, L.J., Alexander, S.C., Alexander, E.C., Gralnick, J.A., Santelli, C.M., Toner, B.M., 2022. Water and rock chemistry inform our understanding of the deep biosphere: case study in an archaean banded iron formation. Front. Microbiol. 10, 1–17. https://doi.org/10.3389/feart.2022.803250.
- Sheik, C.S., Badalamenti, J.P., Telling, J., Hsu, D., Alexander, S.C., Bond, D.R., Gralnick, J.A., Sherwood Lollar, B., Toner, B.M., 2021. Novel microbial groups drive

productivity in an Archean iron formation. Front. Microbiol. 12, 1–21. https://doi.org/10.3389/fmicb.2021.627595.

- Sherwood Lollar, B., Frape, S.K., Fritz, P., Macko, S.A., Welhan, J.A., Blomqvist, R., Lahermo, P.W., 1993a. Evidence for bacterially generated hydrocarbon gas in Canadian shield and Fennoscandian Shield rocks. Geochim. Cosmochim. Acta 57, 5073–5085. https://doi.org/10.1016/0016-7037(93)90609-Z.
- Sherwood Lollar, B., Frape, S.K., Weise, S.M., Fritz, P., Macko, S.A., Welhan, J.A., 1993b. Abiogenic methanogenesis in crystalline rocks. Geochim. Cosmochim. Acta 57, 5087–5097. https://doi.org/10.1016/0016-7037(93)90610-9.
- Sherwood Lollar, B., Heuer, V.B., McDermott, J., Tille, S., Warr, O., Moran, J.J., Telling, J., Hinrichs, K.U., 2021. A window into the abiotic carbon cycle – acetate and formate in fracture waters in 2.7 billion year-old host rocks of the Canadian Shield. Geochim. Cosmochim. Acta 294, 295–314. https://doi.org/10.1016/j.gca. 2020.11.026.
- Sherwood Lollar, B., Westgate, T.D., Ward, J.A., Slater, G.F., Lacrampe-Couloume, G., 2002. Abiogenic formation of alkanes in the earth's crust as a minor source for global hydrocarbon reservoirs. Nature 416, 522–524. https://doi.org/10.1038/ 416522a.
- Simkus, D.N., Slater, G.F., Sherwood Lollar, B., Wilkie, K., Kieft, T.L., Magnabosco, C., Lau, M.C.Y., Pullin, M.J., Hendrickson, S.B., Wommack, K.E., Sakowski, E.G., van Heerden, E., Kuloyo, O., Linage, B., Borgonie, G., Onstott, T.C., 2016. Variations in microbial carbon sources and cycling in the deep continental subsurface. Geochim. Cosmochim. Acta 173, 264–283. https://doi.org/10.1016/j.gca.2015.10. 003.
- Sleator, R.D., Hill, C., 2001. Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol. Rev. 26, 49–71. https://doi.org/10. 1111/j.1574-6976.2002.tb00598.x.
- Stolper, D.A., Lawson, M., Davis, C.L., Ferreira, A.A., Santos Neto, E.V., Ellis, G.S., Lewan, M.D., Martini, A.M., Tang, Y., Schoell, M., Sessions, A.L., Eiler, J.M., 2014. Formation temperatures of thermogenic and biogenic methane. Science 344, 1500–1503. https://doi.org/10.1126/science.1254509.
- Sun, J., Mausz, M.A., Chen, Y., Giovannoni, S.J., 2019. Microbial trimethylamine metabolism in marine environments. Environ. Microbiol. 21, 513–520. https:// doi.org/10.1111/1462-2920.14461.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., Horikoshi, K., 2008. Cell proliferation at 122 °C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. Proc. Natl. Acad. Sci. 105, 10949–10954. https://doi. org/10.1073/pnas.0712334105.
- Teske, A., Hinrichs, K.-U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S.P., Sogin, M.L., Jannasch, H.W., 2002. Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl. Environ. Microbiol. 68, 1994–2007. https://doi.org/10.1128/AEM.68.4.1994-2007. 2002.
- Thompson, A., 2015. A Hydrothermal Model for Metasomatism of Neoarchean Algoma-Type Banded Iron Formation to Massive Hematite Ore at the Soudan Mine, NE Minnesota. M.S. Thesis. Univ. Minnesota.
- Thurman, E.M., 1985. Organic Geochemistry of Natural Waters, 1st edition. Springer, Dordrecht.

- Tissot, B.P., Welte, D.H., 1984. Petroleum Formation and Occurrence, 2nd edition. Springer-Verlag, Berlin.
- Trembath-Reichert, E., Morono, Y., Ijiri, A., Hoshino, T., Dawson, K.S., Inagaki, F., Orphan, V.J., 2017. Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds. Proc. Natl. Acad. Sci. 114, E9206–E9215. https://doi.org/10.1073/pnas.1707525114.
- Vigneron, A., L'Haridon, S., Godfroy, A., Roussel, E.G., Cragg, B.A., Parkes, R.J., 2015. Evidence of active methanogen communities in shallow sediments of the Sonora Margin cold seeps. Appl. Environ. Microbiol. 81, 3451–3459. https://doi.org/10. 1128/AEM.00147-15.
- Wang, D.T., Gruen, D.S., Sherwood Lollar, B., Hinrichs, K.-U., Stewart, L.C., Holden, J.F., Hristov, A.N., Pohlman, J.W., Morrill, P.L., Könneke, M., Delwiche, K.B., Reeves, E.P., Sutcliffe, C.N., Ritter, D.J., Seewald, J.S., McIntosh, J.C., Hemond, H.F., Kubo, M.D., Cardace, D., Hoehler, T.M., Ono, S., 2015. Nonequilibrium clumped isotope signals in microbial methane. Science 348, 428431. https://doi.org/10.1126/ science.aaa4326.
- Ward, J.A., Slater, G.F., Moser, D.P., Lin, L.H., Lacrampe-Couloume, G., Bonin, A.S., Davidson, M., Hall, J.A., Mislowack, B., Bellamy, R.E.S., Onstott, T.C., Sherwood Lollar, B., 2004. Microbial hydrocarbon gases in the Witwatersrand Basin, South Africa: implications for the deep biosphere. Geochim. Cosmochim. Acta 68, 3239–3250. https://doi.org/10.1016/j.gca.2004.02.020.
- Warr, O., Giunta, T., Onstott, T.C., Kieft, T.L., Harris, R.L., Nisson, D.M., Sherwood Lollar, B., 2021. The role of low-temperature 180 exchange in the isotopic evolution of deep subsurface fluids. Chem. Geol. 561, 1–26. https://doi.org/10.1016/ j.chemgeo.2020.120027.
- Watkins, A.J., Roussel, E.G., Parkes, R.J., Sass, H., 2014. Glycine betaine as a direct substrate for methanogens (methanococcoides spp.). Appl. Environ. Microbiol. 80, 289–293. https://doi.org/10.1128/AEM.03076-13.
- Whitehill, A.R., Joelsson, L.M.T., Schmidt, J.A., Wang, D.T., Johnson, M.S., Ono, S., 2017. Clumped isotope effects during OH and Cl oxidation of methane. Geochim. Cosmochim. Acta 196, 307–325. https://doi.org/10.1016/j.gca.2016.09.012.
- Xie, H., Dong, G., Formolo, M., Lawson, M., Liu, J., Cong, F., Mangenot, X., Shuai, Y., Ponton, C., Eiler, J., 2021. The evolution of intra- and inter-molecular isotope equilibria in natural gases with thermal maturation. Geochim. Cosmochim. Acta 307, 22–41. https://doi.org/10.1016/j.gca.2021.05.012.
- Young, E.D., Kohl, I.E., Sherwood Lollar, B., Etiope, G., Rumble, D., Li (李姝宁), S., Haghnegahdar, M.A., Schauble, E.A., McCain, K.A., Foustoukos, D.I., Sutclife, C., Warr, O., Ballentine, C.J., Onstott, T.C., Hosgormez, H., Neubeck, A., Marques, J.M., Pérez-Rodríguez, I., Rowe, A.R., LaRowe, D.E., Magnabosco, C., Yeung, L.Y., Ash, J.L., Bryndzia, L.T., 2017. The relative abundances of resolved ¹²CH₂D₂ and ¹³CH₃D and mechanisms controlling isotopic bond ordering in abiotic and biotic methane gases. Geochim. Cosmochim. Acta 203, 235–264. https://doi.org/10.1016/j.gca.2016.12.041.
- Zhuang, G.-C., Lin, Y.S., Bowles, M.W., Heuer, V.B., Lever, M.A., Elvert, M., Hinrichs, K.-U., 2017. Distribution and isotopic composition of trimethylamine, dimethylsulfide and dimethylsulfoniopropionate in marine sediments. Mar. Chem. 196, 35–46. https://doi.org/10.1016/j.marchem.2017.07.007.